ELSEVIER

International Journal of Pharmaceutics 181 (1999) 71–77

international journal of pharmaceutics

Ocular devices for the controlled systemic delivery of insulin: in vitro and in vivo dissolution

Yung-Chi Lee, Samuel H. Yalkowsky *

Department of Pharmaceutical Sciences, College of Pharmacy, University of Arizona, Tucson, AZ 85721, USA

Received 29 September 1998; received in revised form 9 December 1998; accepted 10 December 1998

Abstract

Both in vitro flow-through and in vivo device removal methods were utilized to determine the dissolution rate of insulin from a Gelfoam® based eye device. The dissolution profiles generated by these two methods are comparable. The in vivo data suggests that there is a direct relationship between blood glucose lowering and the rate of release of insulin from the device. The in vitro dissolution results indicate that the release of insulin from the device is flow-rate dependent. The prolonged activity of the insulin is due to the gradual release of insulin from the device which results from the lachrymal system's slow and constant tear production. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Ocular device; Systemic; Insulin; Eye

1. Introduction

The systemic delivery of insulin via the ocular route has been extensively studied in the past few years (Chiou and Chuang, 1989; Yamamoto et al., 1989; Hopper et al., 1991; Pillion et al., 1991; Bartlett et al., 1994a; Sasaki et al., 1994; Morgan and Huntzicker, 1996). The concept behind ocular drug delivery to the systemic circulation is the use of the stable dynamics of the lachrymal system to get the drug to the nasal cavity where absorption is efficient. Insulin eyedrops containing an absorption enhancer have been shown to significantly lower the blood glucose levels of animals (Chiou and Chuang, 1989; Yamamoto et al., 1989; Hopper et al., 1991; Pillion et al., 1991; Bartlett et al., 1994a; Sasaki et al., 1994; Morgan and Huntzicker, 1996). While it is feasible to deliver insulin via the ocular route, eyedrops produce low therapeutic efficacy, short duration of activity, and low bioavailability (Bartlett et al., 1994a; Bartlett et al., 1994b; Pillion et al., 1995; Morgan and

* Corresponding author. Tel.: +1-520-6261289; fax: +1-520-6264063.

E-mail address: yalkowsky@pharmacy.arizona.edu (S.H. Yalkowsky)

PII: S0378-5173(98)00418-9

Huntzicker, 1996). To improve the efficacy of ocular insulin delivery, the use of an eye insert was proposed Yamamoto et al., 1989).

Recently, it has been demonstrated that a Gelfoam® (absorbable gelatin sponge, USP) based device significantly enhances the therapeutic efficacy, the duration of the effect, and the overall bioavailability of insulin (Simamora et al., 1996; Lee et al., 1997a; Lee et al., 1997b). The manufacturing procedure for the gelatin foam insulin device is relatively simple and the required ingredients are inexpensive (Simamora et al., 1996; Lee et al., 1997a; Lee et al., 1997b). Because it is soft and pliable once hydrated, the Gelfoam® device is comfortable; in fact it can be worn with contact lenses. Since it can be easily removed from the eye if desired, the device has a distinct advantage over most other means of insulin delivery. The Gelfoam® device can give rapid blood glucose suppression and uniformly reduce blood sugar levels for up to 10 h (Simamora et al., 1996; Lee et al., 1997a). It can deliver insulin into the systemic circulation without the use of any absorption enhancer, when treated by at least 5% acetic or 1% hydrochloric acid (Lee and Yalkowsky, 1999). Furthermore, no physical signs of eye irritation (i.e. redness, lachrimation, and restlessness) were observed when the device was used in rabbits (Simamora et al., 1996; Lee et al., 1997a; Lee et al., 1997b).

Although the reduced blood glucose levels produced by the device have been extensively studied, they have not been related to its insulin release rate. Since insulin cannot be absorbed until it is dissolved and released from the device, dissolution is a key factor in prolonging its efficacy. This study is designed to investigate the in vivo and in vitro dissolution of zinc insulin from the gelatin based eye device. Since the device can be easily removed at any time, insulin remaining in the device can be quantitated after its removal. This experiment is particularly interesting because biological response (blood glucose lowering) and the amount of unreleased drug in the device can be measured from the same experiment. These results can then be compared to in vitro release rate data.

2. Materials and methods

2.1. Materials

Gelfoam® (absorbable gelatin sponge, USP, size 100) was generously provided by Pharmacia & Upjohn (Kalamazoo, MI). Zinc bovine insulin (28.6 IU/mg) was purchased from Sigma (St. Louis, MO). All other solvents and chemicals were of reagent or HPLC grade and were used as received from commercial suppliers.

2.2. In vitro assay of insulin

For quantitation of insulin, a Beckman System Gold HPLC and data analysis system were used. The HPLC method is modified from Snel et al. (1987) and the column is an Alltech 4.6*150-mm Adsorbosil RP-8 (5 μm) column. The retention time of insulin is about 5 min and the detection limit is about 2 μg/ml.

2.3. Determination of glucose concentration in rabbit blood

The blood glucose concentration of rabbits was determined by the ONE TOUCH® BASIC™ blood glucose meter which was generously provided by Lifescan (Mountain View, CA). The details of its use were described previously (Lee et al., 1997a).

2.4. Eye device fabrication

A Gelfoam® disc of approximately 6 mm diameter and 2 mm thickness was punched from a slab of Gelfoam® sponge with a common hole punch, and 0.2 mg insulin of Zn-insulin was dissolved in a 30-μl solution of 10% (v/v) acetic acid in water. The solution was placed on and sorbed into the Gelfoam® disc. The wet matrices were dried under vacuum for at least 72 h. Placebo devices were also prepared by this method but without insulin.

2.5. In vivo dissolution

Six New Zealand white rabbits weighing approximately 3 kg were used in the in vivo dissolution test. Each subject had a 6-day washout period and a 12-h fast period prior to dosing. The device was removed at 0.5, 1, 2, 3, 4, and 6 h after instillation. The blood samples were collected immediately prior to device removal and at 0.5 and 1 h after removal. The insulin remaining in the device was extracted by 10% acetic acid-water solution and quantitated by HPLC. To confirm that there is no interference from either gelatin or tear enzymes in the HPLC assay, two placebo devices were instilled into two rabbits. The placebo devices were removed at 6 h post instillation and assayed.

2.6. In vitro dissolution

Some of the reported in vitro dissolution methods for ophthalmic formulations were described by Stevens et al. (1992). A flow through dissolution method, modified from Grass et al. (1984) is used and is shown in Fig. 1. A Swinnex (Millipore) disc filter holder, 9 mm i.d., with an internal volume of approximately 350 μl was used as a dissolution chamber. The Gelfoam® device with or without insulin was placed on the top of the filter. The chamber was closed and pH 7.4 Sorensen phosphate buffer (Deardorff, 1975) at room temperature was passed at 10, 30, or 100 μl/min through the chamber by means of an infusion pump (Syringe Infusion Pump 22, Harvard Apparatus, South Natick, MA). Samples were collected and assayed by HPLC to obtain the amount of insulin released from the device as a function of time.

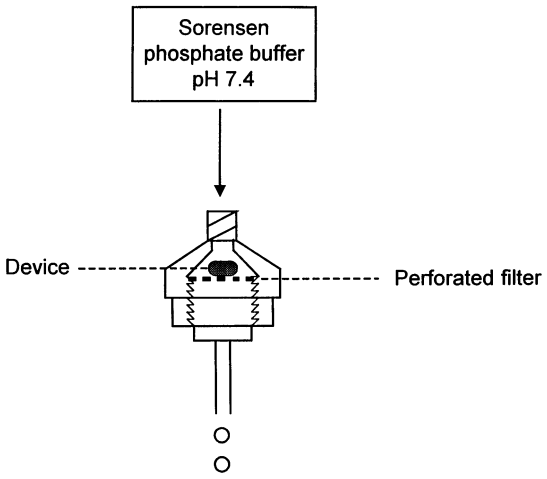

Fig. 1. In vitro flow-through apparatus for insulin dissolution test. This method is modified from Grass et al. (1984).

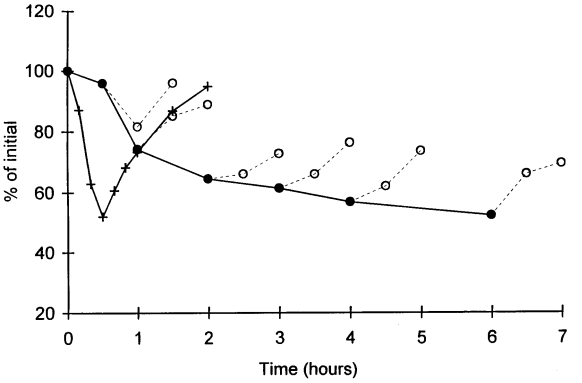

Fig. 2. Mean blood glucose levels before (●) and after (○) device removal and eyedrop (+) administration. Each data point represents $n = 6$ for eye device and $n = 3$ for eyedrop. The averages of standard deviations are all less than 15% and they are not shown. The eye device contains 0.2 mg Zn-insulin and the eyedrop contains 1.0 mg Na-insulin with 20 μg Brij-78. Note that the data of eyedrop was obtained from Lee et al. (1997b).

3. Results

The blood glucose lowering in rabbits before (●) and after (○) device removal is displayed in Fig. 2. For clarity, the standard deviations (all of which are less than 15%) are not shown. As can be seen in the figure, the glucose lowering ceased after each device was removed and was followed by a gradual return to the baseline level. In other words, the device ceases to control blood glucose after it is removed and there is no depot effect. The figure also shows that the blood glucose returning profile following device removal is similar to that generated by eyedrop (+) administration as reported by Simamora et al. (1996).

Fig. 3A shows the percent of insulin remaining in the device after its removal from the eye. These data are from the same devices that are represented in Fig. 2. It is clear that insulin is gradually released in vivo from the device for at least 6 h. After the last removal at 6 h post instillation, 13% percent of the insulin remained in the device. The in vitro flow-through dissolution profiles of the insulin device are shown in Fig. 3B for three flow rates. The dissolution profile generated by this method at the flow-rate of 10 μl/min (upper curve, Δ) is clearly similar to that obtained by in vivo method. At this flow-rate, insulin was slowly released from the device for at least 6 h, after which, 10% of the original insulin still remained in the device. Also, it can be seen from this figure that insulin is released much faster as the flow-rate is increased from 10 to 30 or 100 μl/min.

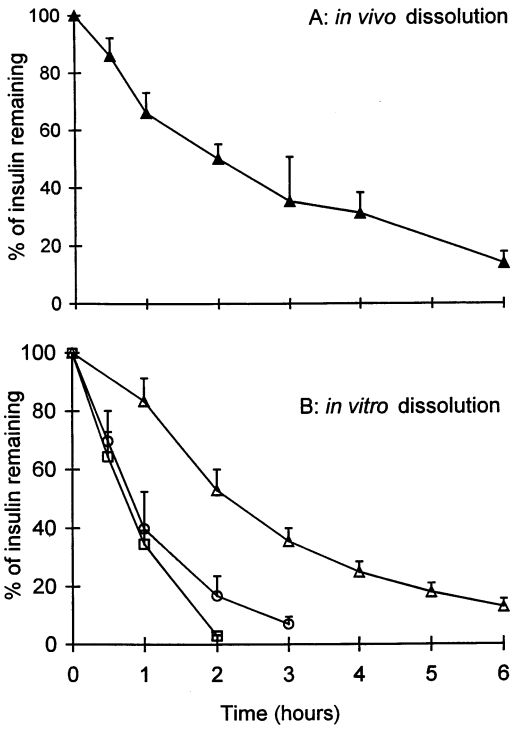

Fig. 3. Dissolution profiles generated by in vivo and in vitro.(A) In vivo device removal dissolution. Each data point represents $n = 6 \pm$ S.D. except the last data point which represents $n = 5 \pm$ S.D. (B) In vitro flow-through dissolution test at different flow-rates: 10 μl/min (Δ); 30 μl/min (○); 100 μl/min (□). Each data point represents $n = 6 \pm$ S.D. for 10 μl/min and $n = 3 \pm$ S.D. for both 30 and 100 μl/min.

4. Discussion

The uniform blood glucose levels indicated by the filled circles in Fig. 2 agree with the results of previous studies in which the glucose levels of rabbits are maintained at about 60% of initial for several hours (Lee et al., 1997b; Lee and Yalkowsky, 1999). However, after the device is removed, the blood glucose levels stop decreasing and begin to return to normal level as indicated by the open circles. The rate of this return is similar to what has been observed for eyedrops.

Since the absorption of insulin is dependent upon its dissolution from the device, the onset time (i.e. the time when the blood glucose levels are lower than 80% of initial) of the device is longer than that of an eyedrop (Simamora et al., 1996). More than 15 different insulin formulations have been tested in more than 60 rabbits. The average time for these formulations to develop blood glucose levels that fall below 80% of baseline is approximately 1 h after instillation (Simamora et al., 1996; Lee et al., 1997a; Lee et al., 1997b; Lee and Yalkowsky, 1999). This is an important advantage of the proposed device because it can prevent the mass entry of insulin and the corresponding precipitous drop in blood glucose that is commonly observed with eyedrops. Furthermore, if blood glucose levels become too low, the device can be easily removed.

Ophthalmic formulations are normally instilled into the conjunctival sac, which is a semi-open environment from which specimens can be easily removed. By taking advantage of this, several techniques have been used to assess the in vivo dissolution of ophthalmic preparations such as sampling tears via capillary (Ding et al., 1992; Jones et al., 1997), cotton swab (Ding et al., 1992), porous polyester rods, (Jones et al., 1997), or filter paper as well as by device removal (Sasaki et al., 1993). In this study, the later technique is applied because the device remains intact for several hours after instillation. As can be seen from Fig. 3A, the in vivo release rate of insulin from

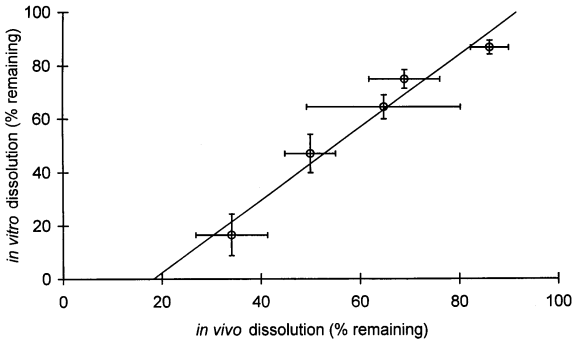

Fig. 4. Correlation of in vivo and in vitro dissolution.

the device is fairly slow and lasts for more than 6 h. This insulin release profile is reflected in the blood glucose lowering profile of rabbits shown in Fig. 2, i.e. the glucose levels of rabbits are maintained in a relatively uniform manner until device removal. These results again confirm that systemic absorption of insulin is governed by the dissolution of insulin from the device and not by a depot effect.

It should be noted that the dissolution profile generated by this method is not a continuous curve and each time point represents a single dose. The fact that all of the variation at $n = 6$ generated in the proposed method is less than 15% implies that device removal is a reliable and precise method for the in vivo dissolution test.

The in vitro dissolution data, displayed in Fig. 3B, has been generated by the proposed flow-through dissolution method. The in vitro dissolution profile produced by this method at the flow-rate at 10 μl/min is similar to that of the in vivo device removal method. While the hydrodynamics around the device in the lower cul-de-sac of the rabbit are certainly different from those described in Fig. 1, the release profiles are similar in magnitude as well as in shape. Fig. 4 shows that there is a linear relationship between the data generated by these two dissolution methods. This confirms the validity of the in vitro dissolution technique for designing devices for in vivo use. The in vitro dissolution data also indicates that the release of insulin from the device is fairly slow but is flow-rate dependent.

The data in Fig. 3A and B (flow-rate at 10 μl/min) are plotted along with theoretical curves for zero order, square root of time, and first order release in Fig. 5. It is clear that both the in vivo and in vitro release profiles are fit comparably well with square root of time or first order kinetics. Although there are not sufficient data to distinguish between these patterns, the dissolution of insulin from the proposed device is steady and, further, it controls the blood glucose levels of rabbits in a uniform manner for over 8 h. It is known that the lachrymal system produces and eliminates tears at an approximately constant 1 μl/min (Lee and Robinson, 1986). Therefore, the prolonged release and activity of insulin may be simply governed by the flow rate of tears through the lower cul-de-sac.

5. Conclusions

Both in vivo device removal and in vitro flow-through dissolution methods are used to investigate the dissolution of insulin from an ocular device. These two methods are easy to perform and the dissolution profiles generated by them are quite similar. Most importantly, these two tests along with the blood glucose lowering response on rabbits verifies that prolonged activity of the insulin ocular device is the result of the gradual dissolution of insulin from the device.

Acknowledgements

This project was partially financial supported by the Yuma Friends of the Arizona Health Sciences Center Young Investigator Research Grant and the Graduate Student Final Project Research Grant, Graduate School, University of Arizona. We would like to thank the Pharmacia & Upjohn Company, (Kalamazoo, MI) for providing samples of Gelfoam® sponge and the Lifescan Company (Mountain View, CA) for supplying the ONE TOUCH® BASIC™ blood glucose monitoring system.

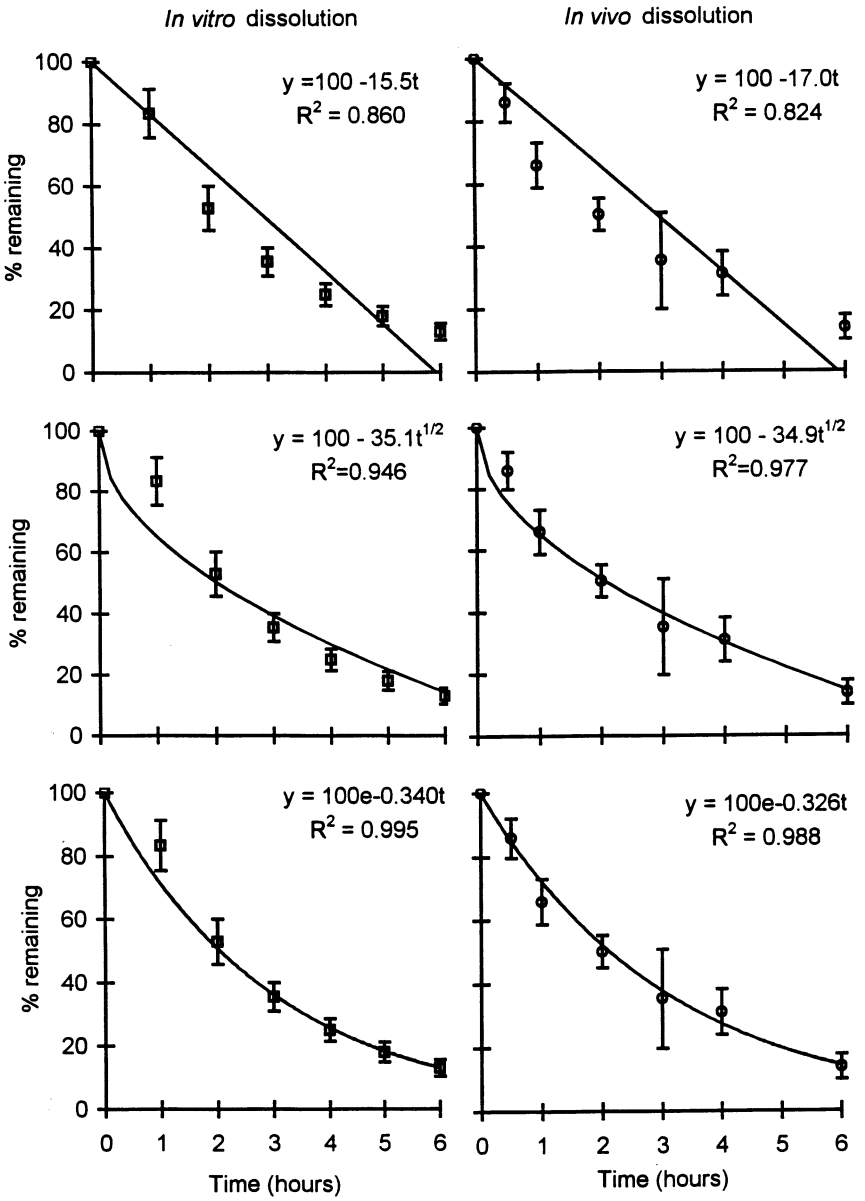

Fig. 5. Comparison of the in vitro (□) and in vivo (○) dissolution to the theoretical curves (–): zero order (top); square root of time (middle); first order (bottom).

References

Bartlett, J.D., Slusser, T.G., Turner-Henson, A., Singh, K., Atchison, J.A., Pillion, D.J., 1994a. Toxicity of insulin administration chronically to human eye in vivo. J. Ocul. Pharmacol. 10, 101–107.

Bartlett, J.D., Turner-Henson, A., Atchison, J.A., Woolley, T.W., Pillion, D.J., 1994b. Insulin administration to the eyes of normoglycemic human volunteers. J. Ocul. Pharmacol. 10, 683–690.

Chiou, G.C.Y., Chuang, C.Y., 1989. Improvement of systemic absorption of insulin through eyes with absorption enhancers. J. Pharm. Sci. 78, 815–818.

Deardorff, D.L., 1975. Ophthalmic preparations. In: Osol, A. (Ed.), The Remington's Pharmaceutical Sciences, 15th ed. Mack, Easton, pp. 1488–1508.

Ding, S., Chen, C.-C., Salome-Kesslak, R., Tang-Liu, D.D.S., Himmelstein, K.J., 1992. Precorneal sampling techniques for ophthalmic gels. J. Ocul. Pharmacol. 8, 151–159.

Grass, G.M., Cobby, J., Makoid, M.C., 1984. Ocular delivery of pilocarpine from erodible matrices. J. Pharm. Sci. 73, 618–621.

Hopper, P.E., Murphy, C.J., Feldman, E.C., Nelson, R.W., Bottoms, G.D., Franti, C.E., 1991. Serum glucose and insulin responses to an insulin-containing ophthalmic solution administered topically in clinically normal cats. Am. J. Vet. Res. 52, 903–907.

Jones, D.T., Monroy, D., Pflugfelder, S., 1997. A novel method of tear collection: comparison of glass capillary micropipettes with porous polyester rods. Cornea 16, 450–458.

Lee, V.H.L., Robinson, J.R., 1986. Review: topical ocular drug delivery: recent developments and future challenges. J. Ocul. Pharmacol. 2, 67–108.

Lee, Y.-C., Yalkowsky, S.H., 1999. Ocular devices for the controlled systemic delivery of insulin II: enhancement by acid treated Gelfoam® (in preparation).

Lee, Y.-C., Simamora, P., Yalkowsky, S.H., 1997a. Effect of Brij-78 on systemic delivery of insulin from an ocular device. J. Pharm. Sci. 86, 430–433.

Lee, Y.-C., Simamora, P., Yalkowsky, S.H., 1997b. Systemic delivery of insulin via an enhancer-free ocular device. J. Pharm. Sci. 86, 1361–1364.

Morgan, R.V., Huntzicker, M.A., 1996. Delivery of systemic regular insulin via the ocular route in dogs. Ocul. Pharmacol. 12, 515–526.

Pillion, D.J., Bartlett, J.D., Meezan, E., Yang, M., Crain, R.J., Grizzle, W.E., 1991. Systemic absorption of insulin delivered topically to the rat eye. Invest. Ophthalmol. Vis. Sci. 32, 3021–3027.

Pillion, D.J., Recchia, J., Wang, P., Marciani, D.J., Kensil, C.R., 1995. DS-1, a modified quillaja saponin, enhances ocular and nasal absorption of insulin. J. Pharm. Sci. 84, 1276–1279.

Sasaki, H., Tei, C., Nishida, K., Nakamura, J., 1993. Drug release from an ophthalmic insert of a beta-blocker as an ocular drug delivery system. J. Control. Release 27, 127–137.

Sasaki, H., Tei, C., Yamamura, K., Nishida, K., Nakamura, J., 1994. Effect of preservatives on systemic delivery of insulin by ocular instillation in rabbits. J. Pharm. Pharmacol. 46, 871–875.

Simamora, P., Lee, Y.-C., Yalkowsky, S.H., 1996. Ocular device for the controlled systemic delivery of insulin. J. Pharm. Sci. 85, 1128–1130.

Snel, L., Damgaard, U., Mollerup, I., 1987. HPLC quantification of rDNA polypeptides like insulin precursors produced in yeast. Chromatographia 24, 329–332.

Stevens, L.E., Missel, P.J., Lang, J.C., 1992. Drug release profiles of ophthalmic formulations. 1. Instrumentation. Anal. Chem. 64, 715–723.

Yamamoto, A., Luo, A.M., Dodda-Kashi, S., Lee, V.H.L., 1989. The ocular route for systemic insulin delivery in the albino rabbit. J. Pharm. Exp. Ther. 249, 249–255.