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# THE PRODUCTION AND EVALUATION OF ORALLY ADMINISTERED INSULIN NANOPARTICLES

R.C. Oppenheim[+*], N.F. Stewart[+], L. Gordon[+], and H.M. Patel[Ø]

[+] School of Pharmaceutics
Victorian College of Pharmacy Ltd.,
381 Royal Parade,
Parkville 3052
AUSTRALIA

[*] For correspondence

[Ø] Department of Biochemistry
Charing Cross Hospital Medical School,
University of London.
Fulham Palace Road,
Hammersmith,
London, W6 8RF,
ENGLAND.

In an attempt to overcome the proteolytic degradation and poor absorption of orally administered insulin, nanoparticles were made from insulin. These 200 nm particles were made from a Neutral Insulin Injection (Actrapid, Novo) by desolvation and crosslinking with glutaraldehyde. The purified nanoparticles were found to be absorbed from the intestinal tract of mice as well as normal and diabetic rats. The blood glucose concentrations in some animals could be reduced to about 15 to 20% of the starting level, 3 hours after administration of between 35 and 70 mg of nanoparticles per 100 g body weight. The nanoparticles appear to exert a slower but more pronounced response than similarly administered Actrapid although no dose response relationship was established. The doses of nanoparticles needed preclude the development of a commercially viable product.

## INTRODUCTION

Proteinaceous hormones such as insulin can only be administered effectively by injection because they are degraded by proteolytic digestion in the gastro-intestinal tract when taken orally. Any residual intact insulin molecule that escapes the digestion would be expected to have a low rate of passive diffusion through gut membranes because of the size of the molecule. However, there are a number of reports[1-3] indicating that insulin can be absorbed in a physiologically active form from the intestines of mammals in the presence of proteolytic inhibitors or certain agents which facilitate absorption in the intestine. The relative activity of orally administered insulin when compared to intravenous administration appears to be less than 2%[3,4].

A variety of formulation procedures have been prepared to increase the effectiveness of oral delivery and absorption of active insulin from the buccal cavity, the gastro-intestinal tract, or the nasal mucosa. These include water-in-oil-in-water emulsions[5-7] and encapsulation of insulin in liposomes[8,9]. These delivery procedures are developed to protect insulin from proteolytic digestions and to facilitate the absorption of the hormone from the gastro-intestinal wall. Irrespective of the detailed mechanism of 'active' transport of such carriers across the intestinal wall, these delivery systems do not produce a consistent hypoglycaemic effect and their effect is not dose dependent. Moreover, the preparations[5-9] have a short shelf life.

Recently Morimoto et al[10] have demonstrated the possibility of rectal administration of insulin in polyacrylic acid aqueous gel. Mesikha[11] used insulin 5U/kg body weight in suppositories

composed of polyoxyl-30 -oleate, Tween 80 with a cocoa butter base on normal and alloxan diabetic rabbits and observed the reduction in the blood glucose by 30-50% for up to 3 h. However this route is not very popular and the effect of long-term exposure of large doses of the permeability increasing surfactant is unknown. Insulin as insulin hydrochloride mixed with a vasodilator in a tablet form has been reported to be absorbed from the buccal cavity[12], but this work has not been followed up.

Hirai et al[4] have shown that insulin administered to the nasal mucosa of rats causes a reduction in blood glucose at doses only ten times greater than that needed with intravenous administration. The variability of the state of the mucosa must cast doubts on the reliable passive absorption of such a large molecule.

To overcome some of the difficulties of the delivery system described earlier[5-9], we investigated the possibility of oral administration of solid proteinaceous nanoparticles in rats and mice. Nanoparticles are cross-linked, non-porous aggregates of naturally occurring macromolecules. They are submicron in size and stored as a freeze dried powder[13-15]. However, instead of using natural proteins such as albumin or gelatin as the base with drugs incorporated into the nanoparticle, it was proposed to make nanoparticles out of insulin alone. This paper described the production and in vivo evaluation of insulin nanoparticle absorption from the gastro-intestinal tract of rodents.

## MATERIALS AND METHOD

### Nanoparticle Manufacture

Batches of insulin nanoparticles were prepared from 10 ml vials of Neutral Insulin Injection (Actrapid, 80U/ml, Novo).

Sephadex G-50m (Pharmacia South Seas Pty. Ltd., Sydney) and glutaraldehyde 25 per cent w/v aqueous solution (Koch-Light Laboratories, England) were used as received. All other chemicals used were AR grade.

A Nepho-colorimeter, Model 9 (Coleman Instruments Corporation, U.S.A.) was used to monitor changes in intensity of scattered light during the desolvation/resolvation steps in the nanoparticle manufacture. A Silverson laboratory homogenizer fitted with a microhead, an Isco Fraction Collecter (Model 1200 Pup) and a Dynavac $FD_2$ Freeze Drier were also used.

Typically one 10 ml vial of Actrapid equilibrated at 25°C required 0.7 ml of 0.1M hydrochloric acid to cause a rapid rise in nephelos number. Addition of about 0.1 ml of 0.1M NaOH reduced the nephelos number to a range suitable for the cross-linking reaction to be undertaken. 0.2 ml of 25% glutaraldehyde solution was added in one aliquot; the system homogenized for 15 seconds every 30 seconds for a total of 4 minutes after which 6 ml of 12% w/v sodium metabisulphite was added, and the same homogenizing pattern continued for a further 6 minutes.

The crude insulin nanoparticles were purified either by passage through a Sephadex G-50m column using 0.04% chlorbutol solution as the eluant or alternatively they can be collected on a 0.25 μm Millipore filter and repeatedly washed with 10 ml lots of distilled water. The desalted purified nanoparticle dispersion was then freeze dried to yield typically 20 mg of a white free flowing powder.

In vivo Evaluation

Two series of experiments were undertaken. In the first series, non-pregnant female Hooded Wistar rats over 150g were

drawn from the Victorian College of Pharmacy Ltd. stocks. After fasting overnight, 0.1 ml of 15% of alloxan monohydrate was delivered per 100g rat subcutaneously to the upper thigh of conscious animals. The rats were then allowed food and drink ad lib. and were used between one to two weeks after injection.

Rats were anesthetized with intraperitoneally injected sodium pentobarbitone using 45 mg/kg for normal rats and 60 mg/kg for diabetic rats. Insulin preparations were usually administered 30 minutes after induction. The tail vein was used for the 100 µl intravenous injection and the upper thigh for 150 µl for intramuscular injection. The upper part of the small intestine was exposed and the intestine was then tied off below the stomach. The intestine was nicked slightly distal to the tie and a blunt needle was used to administer the 1.5 ml injection to the gut.

Blood glucose levels before and after administration of the insulin products were determined by sampling from the tail vein. The samples were stored in small plastic vials containing sodium fluoride and the glucose levels determined within 20 minutes on a calibrated Yellow Springs Glucose analyser.

In the second series of experiments, insulin preparations were introduced intragastrically by stomach tube into rats and mice. This overcame any complications caused by the surgical procedure and anesthetic and gives more realistic conditions for the desired type of administration. Appropriate volume of Actrapid (80U/ml) or a 66.6 mg nanoparticle in 1 ml normal saline solution were adminstered. Normal Wistar rats of either sex (200-250 g body weight) drawn from Charing Cross Hospital stocks were starved overnight and placed in a restraining cage two hours

before starting the experiments. Rats of between 130-150g body weight were made diabetic by intravenous injection of streptozotocin (Upjohn) freshly prepared in 0.01 M citrate buffer of pH 4.5 at a dose of 65 mg/kg body weight. The rats were used one week after the streptozotocin injection. Normal outbred mice (T/O strain) of body weight 18-22g were used after being fasted overnight.

Blood samples were taken from the tails of the rats and by ophthalmic venous puncture in mice. Blood glucose levels were determined by deproteinizing 50 µl of blood by adding 0.3 ml of 0.3MNaOH and 0.6 ml of 10% Zinc Sulphate. A further 50 µl of distilled water was added to make up 1 ml. After centrifuging on a bench centrifuge for 5 minutes, the supernatant was collected and between 0.2 and 0.5 ml was diluted to 1 ml with distilled water for the glucose estimation.

The diluted sample was mixed with a 2 ml of a glucose oxidase reagent (from Boehringer) and incubated for one hour at 37°C. Following addition of 2 ml of 18 N $H_2SO_4$, the colour developed was read at 540nm on a Corning Spectrophotometer 256[16].

## RESULTS

### Nanoparticle Manufacture

Figure 1 shows a typical scanning electron microscope of a typical batch of 200 nm insulin nanoparticles. They readily dispersed in water or normal saline to form a 10% colloidal solution.

### In Vivo Evaluation

Doses of insulin from nanoparticles or Actrapid are expressed in milligrams per g body weight for easy comparison, 24 IU/mg being used as the conversion factor for Actrapid. Results are expressed

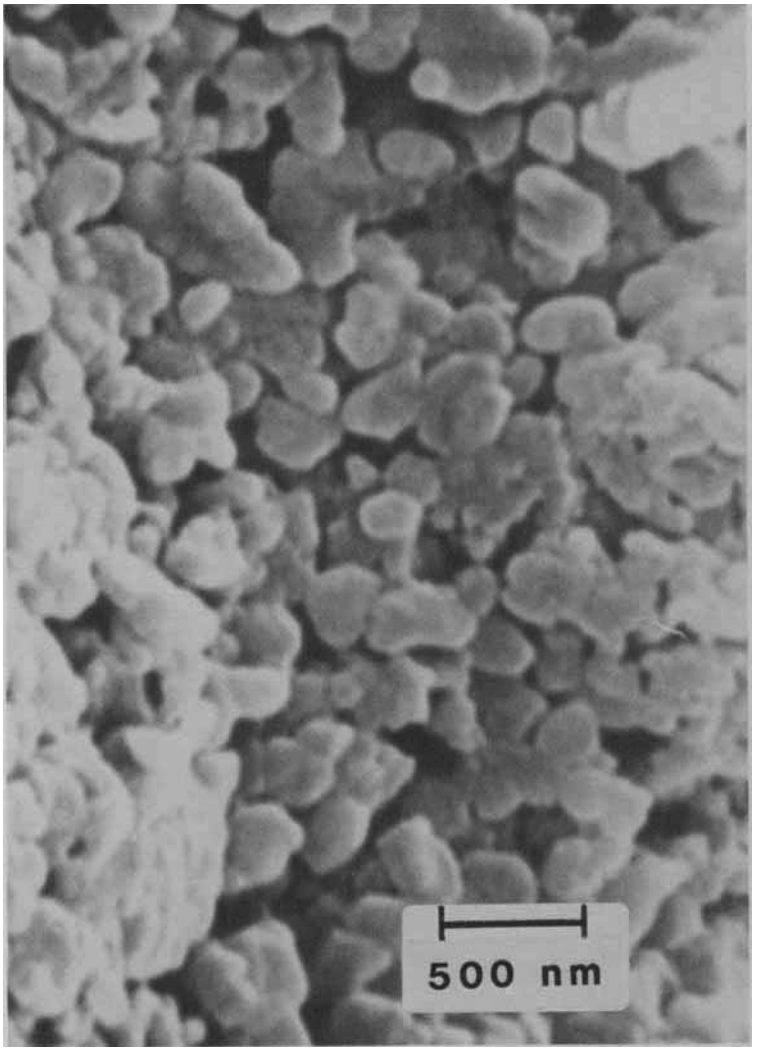


Fig 1

Scanning electron micrograph of insulin nanoparticles

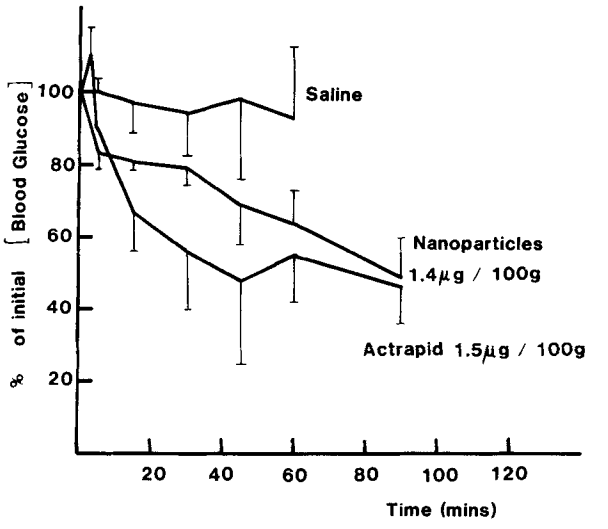


Fig 2 I V administration of saline, nanoparticles and Actrapid to normal rats. Typical standard deviations shown.

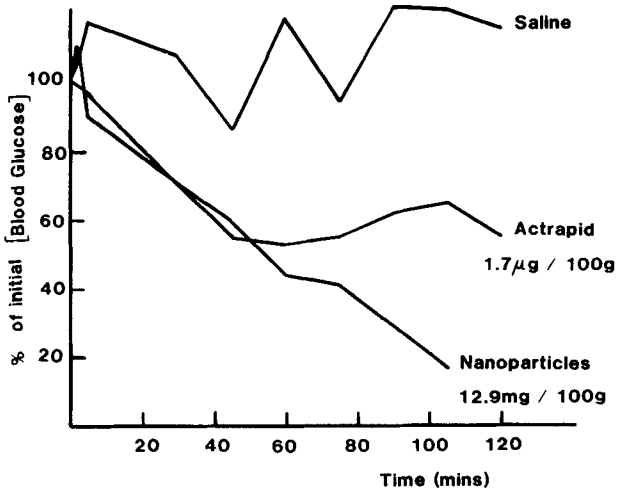


Fig 3 IM administration of saline, nanoparticles and Actrapid to normal rats.

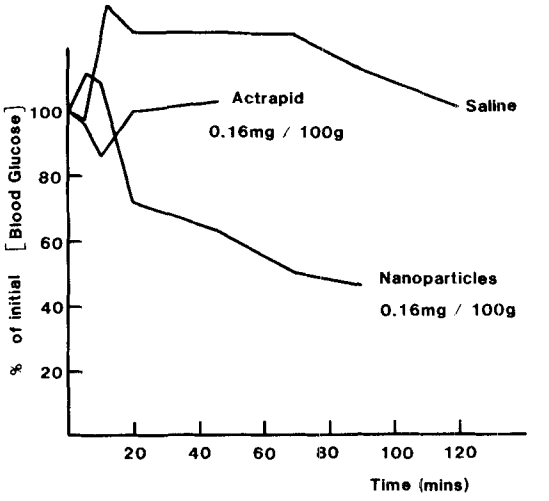


Fig 4

Direct injection of saline, nanoparticles and Actrapid into small intestine of normal rats.

as a percentage of the blood glucose concentration before treatment. In normal animals this reference level was between 70-80 mg per 100 ml and that in diabetic rats was 200-240 mg per 100 ml. The Figures give the results of the first set of experiments, the Tables those for the second set of experiments.

Figure 2 gives the results for intravenous injection to normal rats. Each result is the average of at least four rats. Figure 3 gives the results of intramuscular injection. Each result is the average of at least two rats. The results of administering insulin preparations directly into the small intestines or normal and diabetic rats are given in Figures 4 and 5 respectively. By repeating the experimental results shown typically in Figure 2, it was found that the insulin nanoparticles used in the second series of experiments had a potency of about 5 IU/mg.

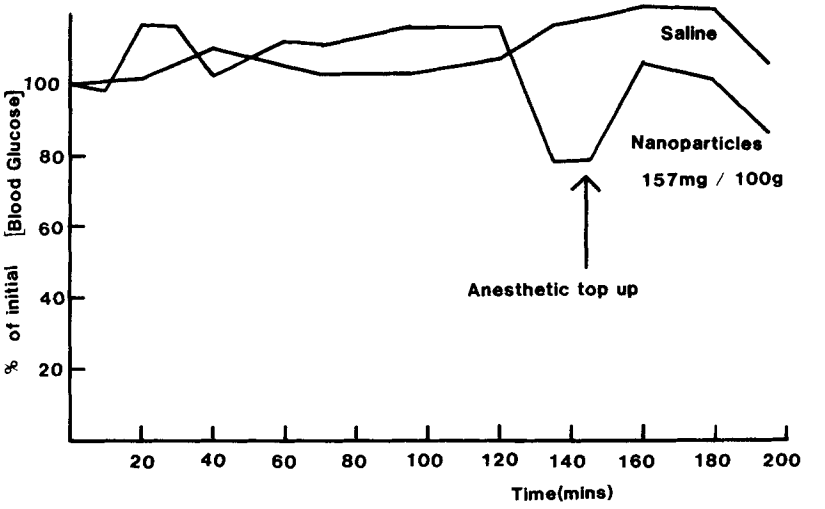


Fig 5
Direct injection of saline and nanoparticles into small intestine of diabetic rats.

TABLE 1

Oral administration to normal mice

| Dose/100 g body weight | Mouse No. | Blood Glucose Level % of Initial Value Time: 2 hr | 3 hr |
|---|---|---|---|
| 185 mg nanoparticles | 1 | 69.0 | 55.4 |
| | 2 | 80.0 | 57.0 |
| | 3 | 64.6 | 67.0 |
| 275 mg nanoparticles | 4 | 97.0 | 81.0 |
| | 5 | 113.0 | 144.0 |
| | 6 | 94.2 | 128.0 |

TABLE 2

Oral administration to normal rats

| Dose/100 g body weight | Rat No. | Blood Glucose Level % of Initial Value Time: 1 hr | 2 hr | 3 hr |
|---|---|---|---|---|
| Saline | 1 | 85.0 | 111.0 | 108.0 |
| | 2 | 95.0 | 114.0 | 114.0 |
| Actrapid 2.4 mg | 3 | 107.0 | 100.0 | 106.0 |
| | 4 | 115.0 | 90.0 | 104.0 |
| | 5 | 125.0 | 124.0 | 120.0 |
| Actrapid 2.22 mg | 6 | 41.2 | 40.0 | 17.5* |
| | 7 | 44.2 | 81.4 | 25.7 |
| Nanoparticles 70 mg | 8 | 57.0 | 39.0 | 15.0* |
| Nanoparticles 60 mg | 9 | 63.2 | 86.8 | 40.7 |
| | 10 | 80.5 | 71.6 | 13.1* |
| | 11 | 62.8 | 47.1 | 24.3* |
| | 12 | 63.5 | 60.7 | 57.5 |
| Nanoparticles 35 mg | 13 | 61.0 | 34.0 | 16.0* |
| Nanoparticles 17.5 mg | 14 | 126.0 | 134.0 | 145.0 |

* rats developed hypoglycaemic shock

TABLE 3

Oral administration to diabetic rats

| Dose/100 g body weight | Rat No. | Blood Glucose Level % of Initial Value Time: 1 hr | 2 hr | 3 hr |
|---|---|---|---|---|
| 2 ml saline | 1 | 93.4 | 101.0 | 102.0 |
| | 2 | 97.5 | 103.0 | 102.0 |
| | 3 | 93.0 | 92.7 | 111.0 |
| Actrapid 2.22 mg | 4 | 63.6 | 58.6 | 55.0 |
| | 5 | 38.0 | 79.0 | 58.0 |
| Nanoparticles 60 mg | 6 | 50.0 | 29.6 | 28.1 |
| | 7 | 50.0 | 35.3 | 72.0 |
| | 8 | 71.0 | 36.5 | 30.0 |
| | 9 | 73.5 | - | 79.7 |
| | 10 | 40.2 | 64.7 | 33.3 |

Tables 1 and 2 show the results of oral administration to normal mice and rats respectively. Table 3 shows those for diabetic rats.

## DISCUSSION

As the nanoparticles are composed of many intermolecularily cross-linked insulin molecules, it is unlikely that they would have significant antigylcaemic activity without breakdown of the nanoparticle to its constituent molecule. Following intravenous administration the main part of this enzymatic breakdown presumably would occur in the liver and so nanoparticle insulin would be expected to have a slower rate of onset than the Actrapid insulin. The results shown in Figure 2 confirm this, as nanoparticles caused reduction in the blood glucose level which is comparable with that observed with Actrapid insulin with a slower rate of onset with nanoparticles than Actrapid.

It is known[17] that intramuscularily administered nanoparticles slowly accumulated in the liver. Figure 3 shows that nanoparticles do have activity when given this way, but the dose needed is quite high. This is consistent with the slowrate of accumulation in the liver possibly coupled with a very slow intramuscular degradation of nanoparticle. Since the main objective of the study was oral administration, the detailed dose and time profiles of intramuscular insulin nanoparticles were not pursued.

To overcome the possibility of acid hydrolysis of nanoparticle in gut, we introduced nanoparticle directly into jejenum of normal and diabetic rats. The response of the animal to the anaesthetic and anaesthetic 'top ups' as well as to the surgery to expose the

small intestine led to wide variations in glucose levels. However, the results given in Figures 4 and 5 show that in some animals at least, the nanoparticle insulin has a hypoglycaemic effect whereas the similarily administered Actrapid does not. Not all rats responded to nanoparticle therapy. When nanoparticles were administered orally in the second series of experiments to normal mice in doses up to 185 mg/100 g of body weight (Table 1) and to normal and diabetic rats in the dose of up to 60 mg/100 g of body weight (Tables 2 and 3) a reduction in the blood glucose level was usually achieved. We also found that Actrapid given orally also sometimes caused a drop in blood glucose levels. However the limited results in Table 1 indicate that at the higher dosage, there was no hypoglycaemic effect. It may be that at higher doses there is some inhibitory effect. In suckling rats and mice, the absorption of protein is markedly inhibited by increase in the intragastric dose of macromolecules[18]. Since the rat results in Table 2 indicate hypoglycaemic shock occurred at doses as low as 35 mg/100 g body weight, it may be that there is some inherent difference in response between rats and mice. The results in both Tables 2 and 3 strongly suggest that nanoparticle insulin administered perorally does reduce the blood glucose levels for periods probably well exceeding the 3 hours allowed by the experimental protocol and at doses lower than that called for in the experimental design.

We believe that further experiments to understand the mechanism and the site of absorption of nanoparticle and modification of nanoparticle preparation may help to minimize the dose and improve the efficiency of absorption. For example nanoparticles made from a mixture of insulin and proteolytic inhibitor such as trasylol [a strong inhibitor of proteolytic enzymes from

gut of man, dog and rodents; (Patel and Ryman, unpublished observation)] administered orally with some natural surface active agents may overcome the problem of insulin degradation and increase the permeability of gastro-intestinal wall.

## CONCLUSIONS

The following conclusions may be drawn from this study:

1 Insulin nanoparticles can be absorbed from the gastro-intestinal tract of mice, normal and diabetic rats.

2 The absorbed nanoparticles are biologically active and reduce the blood glucose concentration of these animals.

3 Orally administered nanoparticles appear to exert a slower but more pronounced response than similarly administered Actrapid.

4 No direct dose response relationship has been demonstrated.

5 Rats appear to respond better than mice to oral nano-particles.

6 Orally administered insulin nanoparticles does not appear to be a commercially viable product.

## ACKNOWLEDGEMENTS

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