Poloxamer/chitosan crosslinked gel as nasal insulin delivery system: *in vitro* characterization and *in vivo* evaluation

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Abstract Summary

New thermosensitive gels that combine the Poloxamer (P), chitosan (CS) and glutaraldehyde (GA), called P-CS/GA gels, were examined to evaluate their properties *in-vitro*. Moreover, with glycine (Gly) addition, the insulin delivery behaviors of P-CS/GA/Gly gels *in-vivo* by nasal administration in diabetic rats were investigated. P-CS/GA gels (33: 1: 0.1, (wt %)) have a swelling ratio of up to 13.2 ± 1.0, which is maintained for approximately 18h. With the addition of a large excess of glycine (Gly) to inhibit crosslinking reactions of residuals of GA, insulin delivery with P-CS/GA/Gly gels significantly reduced the burst release of insulin ($P<0.01$) below those of P or P-CS gels, and markedly sustained insulin release ($P<0.01$) for up to 20 h - which was about six times or more that of P or P-CS gels *in-vitro*. Nasally administered insulin with P, P-CS and P-CS/GA/Gly gels in diabetic rats had hypoglycemic effects which reduced initial blood glucose level by up to 50 % 2 hr after it was administered. Notably, the lowest glucose levels of the rats that had been administered P-CS/GA/Gly gels were maintained for 3 hr. The new P-CS/GA/Gly gels effectively sustained insulin release *in-vitro*, and *in-vivo* nasal insulin delivery, suggesting that they may be potential carriers of insulin for nasal delivery.

Introduction

Poloxamer block copolymers comprise various poly (ethylene oxide) (PEO) and poly (propylene oxide) (PPO) blocks arranged in a tri-block polymer structure; (PEO)-b-(PPO)-b-(PEO). At a LCST, Poloxamer micelles pack in an order that results in a transition of sol to the gel state. [1] As a drug delivery carrier, P gels such as Poloxamer 188 and 407 generally sustain the release of drugs for a short period (such as < 5 hr) in an aqueous environment [2,] since the P gels rapidly in an aqueous environment. The maximum duration of drug release is generally limited by the influx of water into the gels, which dilutes the Poloxamer and turns the gel into the solution state. A simple method which does not need complex chemical modifications of Poloxamer to reduce the dissolution rate and improve the sustained-release characteristic of drugs is sought.

Type I or Type II diabetes mellitus must receive subcutaneous injections of insulin to keep their blood glucose level in the normal range. Nasal insulin administering represents an alternative means of improving the control of blood glucose levels in patients and has been extensively investigated.[3] Chitosan (CS), poly(1, 4 - D - glucosamine), has been widely employed in drug delivery. Moreover, the nasal delivery of insulin in CS solutions has been demonstrated to increase greatly trans-mucosal absorption in rats and sheep.

New P-CS/GA gel is developed which may overcome the shortcoming of the fast dissolution of P gels in aqueous environments. The *in-vitro* insulin delivery behaviors of P-CS/GA and P-CS/GA/Gly gels were examined. The *in-vivo* nasal insulin delivery with P, P-CS and P-CS/GA/Gly gels in diabetic rats was adopted as a model to evaluate possible the hypoglycemic effect and the pharmacological bioavailability of the gels.

Experimental Methods

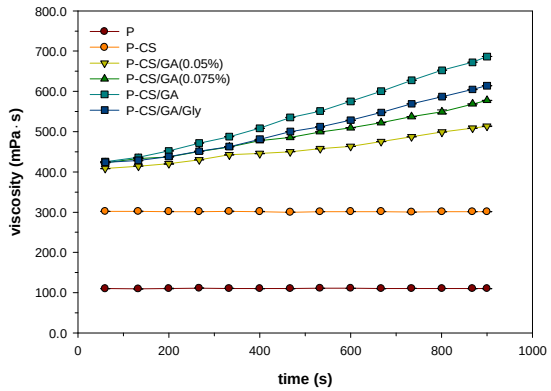
Poloxamer (P) solution contains F127 (18 wt.-%) and F68 (15 wt-%) in DI water, and P-CS solution contained 1 % of CS in P solution. P-CS/GA solutions contain various concentrations of glutaraldehyde (GA) (0.05 ~ 0.1 wt.-% P-CS/GA solutions). P-CS/GA/Gly solution was prepared as that P-CS/GA solution was initially subjected to 10 min of 0.1 wt.-% GA crosslinking reactions, and then large excess amounts of glycine (Gly) was added to the P-CS/GA solution. Bovine insulin was dissolved in Hcl and adjusted by NaOH solution before being transferred into P-CS and P-CS/GA/Gly solution to prepare 100 IU/ml insulin solutions.

The following characteristics were investigated for the solutions or gels: **A**. Viscosity measurements -- 1ml of each solution was added to a cone/plate viscometer by a rheometer with variation of the shear rates from 30 s^{-1} to 300 s^{-1}; **B.** Swelling and dissolution properties of the gels -- 1g of the gel was immersed in a vial of dissolution medium at 37 ^{0}C.The swelling ratio of a gel was defined as the ratio of the increase in the weight of the hydrated gel at each measurement time to the original weight of the gel; **C.** In-Vitro release of insulin from the gels -- the gels were placed in an osmosis membrane with a cut-off MW of 300 kDa and suspended in vials of the dissolution medium at 25 ^{0}C; **D.** In vivo evaluation of intranasal administering of gels in diabetic rats -- diabetic Wistar rats (250-300g) fasted for 24 hrs. For the rats of nasal delivery groups, 30 µl of insulin free P-CS solution, and insulin in P, P-CS and P-CS/GA/Gly solutions at a dosage of 10 IU/kg were loaded into a micro- pipette for nasal administering. The blood samples were taken from the tail vein at every half hour following dosing and the glucose levels were determined using a glucose kit. The relative pharmacological bio- availability (Fr) was calculated as Fr (%) = (AOC_{nasal} / $Dose_{nasal}$) / (AOC_{sc} / $Dose_{sc}$) x 100 % where AOC is defined as the areas over the glucose level / time curves

and below the baseline. Data are presented as mean ± standard deviation.

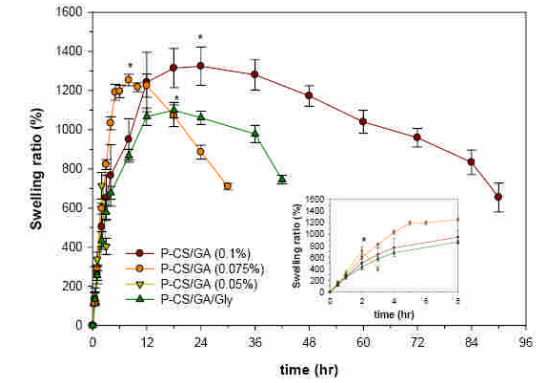
Results and Discussion

Figure 1 plots the viscosities of P-CS and P-CS/GA solutions with various concentrations of GA, respectively, at 30 ^{0}C as a function of time (from 60 s to 900 s).



The viscosity of the P-CS/GA solution increased with time, and the rate of increase of viscosities was positively correlated with the amount of GA in the solution (Fig. 1).

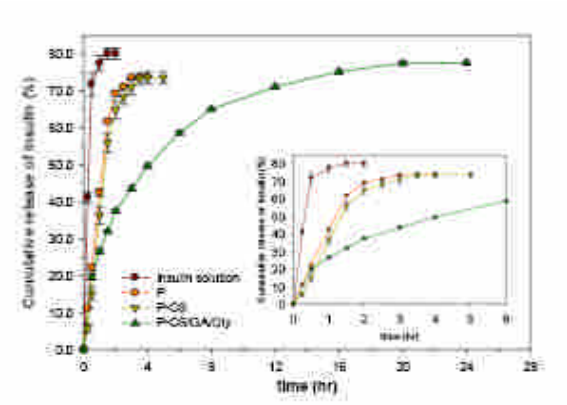
The maximum swelling ratios of the P-CS/GA gels that contained 0.05, 0.075 and 0.1 wt% GA were 7.1 ± 0.7, 12.5 ± 0.3 and 13.2 ± 1.0 (in wt, n=3) times of those of P-CS gels, respectively, although the time to reach the maximum ratio varied with the amount of GA in the gel (Fig. 2).



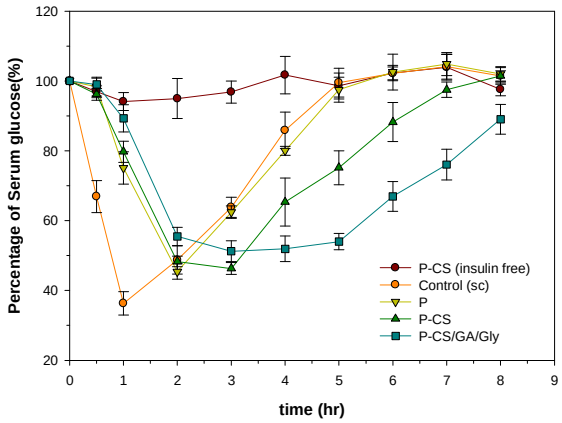
The maximum swelling ratio of the P-CS/GA/ Gly gel was 10.9 ± 0.4, obtained at 18h (Fig.2). In addition, the times required for the dissolution of 50 % of the maximum swelling weights of the gels that contained 0.075 and 0.1 wt% were about 30hr and 90hr, respectively.

Notably, the burst release of insulin from P-CS/GA/Gly gel was significantly reduced ($P<0.01$, n=3) (from 70 % of total release of insulin down to 20 % at 0.5h) and the end of the release of insulin was markedly extended to 20 hr, which is about six or more times the period of insulin release from P or P-CS gels (Fig. 3).

The blood glucose levels of rats following the nasal administering of insulin (10.0 IU/ Kg) in various formulations of gels, and those of the control group, were monitored (Fig.4).



Notably, for the rats that had been nasally administered insulin using P-CS/ GA/Gly gels, C $_{min}$ was also reached at 2 hr and the C $_{min}$ state lasted for 3 hr The Fr (%) of insulin nasally delivered with P, P-CS and P-CS/GA/Gly gels was 9.3 ± 0.9 %, 13.6 ± 1.8 % and 18.0 ± 1.8 % (n=6), respectively, calculated with reference to the result obtained from sc group



Conclusion

New thermosensitive P-CS/GA gels have a swelling ratio of up to 13.2 ± 1.0 , which is maintained for about 18 h. Insulin delivery with P-CS/GA/Gly gels significantly reduced the burst release of insulin compared with those of P or P-CS gels, and substantially sustained insulin release for up to 20 h *in-vitro*. P-CS/GA/Gly gels were employed for the nasal delivery of insulin in diabetic rats that is associated with a highly pro-longed hypoglycemic effect and pro- motes Fr (18.0 ± 1.8 %), suggesting that the gels may be potential carriers of insulin for nasal delivery.

References

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