

## Original Research Article

# Enhancing Effect of Bile Salts on Gastrointestinal Absorption of Insulin

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### Abstract

**Purpose:** To investigate the effect of co-administration of two absorption enhancing bile salts, sodium glycocholate (NaGc) and sodium salicylate (NaSal), on insulin absorption via intestinal targeted delivery system.

**Methods:** Insulin (10 IU/kg), associated with and without absorption enhancers (5 % enhancer solution of NaGc or NaSal), was administered to the duodenum, jejunum, and ileum part of the diabetic rat's gastrointestinal (GI) tract by surgical technique. The insulin absorbed from the GI tract was evaluated by its hypoglycemic effect at 45 and 60 min post-administration.

**Results:** The results showed that insulin formulations containing NaGc or NaSal administered into the duodenum and with little quantity in the jejunum decreased blood glucose levels, compared to the reference formulations ( $p < 0.05$ ). It was also observed that formulations containing cellulose acetate phthalate (CAP) protectors and enhancers may protect insulin during transit through the stomach for 180 min.

**Conclusion:** Thus, the results of this study demonstrate that duodenum-specific delivery of insulin with NaGc and NaSal was achievable by oral administration compared to the other parts of small intestine. Furthermore, NaGc has a greater enhancing effect on duodenal, and to some extent, jejunal absorption of insulin.

**Keywords:** Bile salts, Sodium glycocholate, Sodium salicylate, Insulin, Gastrointestinal administration

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## INTRODUCTION

Insulin is still the principal hypoglycemic medication in diabetes mellitus treatment but administration of these peptide drugs so far have been almost exclusively limited to the parenteral route [1]. Oral administration of insulin is considered to be the most suitable route. Generally, oral administration of peptides gives rise to a very low bioavailability [2-4]. Alternative specific insulin delivery systems have been tried to improve the low availability of insulin following oral administration. Various absorption promoters

such as surfactants, bile salts, chelating agents, fatty acids, protease inhibitor and soy bean trypsin inhibitor have been used for improving the absorption of poorly absorbable drugs such as insulin [2]. The effectiveness of NaGc and NaSal in mucosal absorption of insulin has been reported in several investigations [5,6]. However it was found that the effect of these absorption enhancers in the large intestine was more remarkable than that in the small intestine. Meanwhile, NaGc enhance the permeability of insulin across the intestinal membrane [2,7] but the effectiveness of NaSal to improve oral

absorption of drugs is still unclear. Therefore, in this study NaGc with NaSal were used as model bile salts due to their absorption enhancing effects for insulin [8] and other peptides [9] to overcome the low availability of insulin oral administration.

In this regard, many researchers have investigated alternative routes for insulin administration such as nasal, pulmonary, buccal, oral, colonic and rectal routes. However the colon is considered the most preferred site for the absorption of peptide drugs because of the lower proteolytic activity of enzymes in this region compared to the small intestine [10-13]. However, drug absorption from small intestine is much greater than from the colon; water content of the colon is also much lower [2]. Few studies have focused on the intestine as a potential delivery region for several therapeutic peptides such as insulin. Small intestinal drug delivery would be more effective if one can prevent drug from degradation in the small intestine. This study focused on insulin drug delivery into via parts of the small intestine as a potential delivery site for absorption of protein drugs such as insulin.

The aim of the present study, therefore, was to evaluate the effectiveness of two penetration enhancers to increase gastrointestinal absorption of insulin in order to improve the absorption of orally administered insulin.

## EXPERIMENTAL

### Materials

Insulin (ActRapid Beef, Novo Nordisk) was used as the model drug. Bile salts (enhancers) sodium glycocholate and sodium salicylate was purchased from Fluka (Germany). All other chemicals and solvents were analytical grade and obtained from Sigma (Germany).

### Preparation of formulations

Gelatin microspheres were prepared according to the method of Tabata and Ikada [10]. Briefly, gelatin solution was added drop wise to olive oil at 40 °C under mechanical stirring to give an emulsion, then the temperature was dropped to 4 °C and cold acetone was added for the gelation of gelatin. Microspheres were collected by centrifugation, and washed with acetone and isopropanol, followed by cross-linking with glutaraldehyde in aqueous solution. After washing with purified water, microspheres were freeze dried. Insulin and enhancer solutions were

added to freeze-dried blank microspheres, and then microspheres were incubated in 37 °C water bath for 1 h followed by freeze-drying. Finally, microspheres were coated with thin layer of cellulose acetate phthalate (CAP) via the dipping method.

For preparation of enhancer solutions, 5 g of two enhancers were dissolved in a phosphate buffered saline solutions (PBS) adjusted to pH 7.4. The final enhancer concentration in each formulation was 5 % w/v.

### Animal studies

Male Albino rats (mean weight 166 g; mean age, 4 months) were fasted for more than 8 h before the experiments and then diabetized by injecting 2 % of an alloxane aqueous solution into the tail vein. Blood glucose concentration above 250 mg/100ml was assumed as diabetes. 72 h later, the animals were anesthetized by 50 mg/kg pentobarbital (i.p.). Studies in this article have been carried out in accordance with the guideline of the Animal Ethics Committee Jundishapur University of Medical Sciences, Ahvaz, Iran (ref no. 86-D). The anesthetized animals were prepared for injection by surgical procedure using abdominal incision. The purpose of this invasive method was to describe evidenced-based interventions that may be beneficial for oral-specific delivery. One ml of formulations was administered into stomach, duodenum, jejunum and ileum. The exposed intestinal segments were identified in the following method. The first 10 cm was considered as the duodenum, the next 10 cm was used as the jejunum and the last 10 cm was considered to be the ileum [7]. Dose of insulin in formulations was 10 IU/Kg. The ear vein blood samples were collected into heparinized syringes at 0, 30, 60, 120 and 180 min following stomach administration and 45 and 60 min following intestinal administration. Samples were immediately centrifuged (Clements 2000 Centrifuge, Australia) at 15,000 rpm for 10 min to obtain the plasma fraction (approx 200 µl), which was then kept in ice until determination.

### Blood glucose determination

Glucose with ortho-toluidine in the presence of acetic acid, produce green glucosamine. Three grams of thiourea was mixed with 1.9 L of acetic acid and then 100 ml of ortho-toluidine was added. Prepared ortho-toluidine was stored in amber container [14,15]. For preparation of glucose standard solutions, 200 mg of glucose was added to 100 mL of distilled water and the final solution was diluted to different

concentrations. The stock solution was then diluted to 50, 100, 150, 200 and 250 mg/ 100ml and analyzed in a spectrophotometer (Shimadzu UV 60, Japan) for their absorbency at 630 nm, using the ortho-toluidine method. Blood glucose concentration was measured and compared with standard solutions. Glucose with ortho-toluidine in the presence of acetic acid, produce green glucosamine [14].

### Statistical method

SPSS (version 16) was used for statistical comparisons of control and treatment groups. The presented data were the mean of three experiments. Results were shown as Mean  $\pm$  SEM. Student t-test was performed to compare test results with the control results samples. Values of  $p < 0.05$  were assumed as significant terms.

## RESULTS

Bile salts were compatible with insulin and no precipitation or aggregation was observed during preparation of insulin-bile salts formulations in phosphate buffer with pH 7.4. To assure their stability, the solutions were prepared just prior to the tests. Tables 1 and 2 show the effect of injecting different insulin formulations with or without CAP and enhancers into the stomach of rats on blood glucose concentration. As shown in Table 1, there was little difference between plasma glucose concentration before and following administration of formulations until 120 min, especially for formulations containing NaGc. After this period, however, a decrease in blood glucose concentration was observed.

Figures 1, 2 and 3 show the blood glucose concentration time profile after administration of formulations into different small intestinal parts of anesthetized rats with or without absorption enhancers.

**Table 1:** The effect of three administered formulations into rats stomach on blood glucose level (mg/100 ml) (n = 5, Mean  $\pm$  SEM)

Formulation	Time (min)				
	0	30	60	120	180
Insulin+CAP+NaSal	367 $\pm$ 34.3	362.2 $\pm$ 36	342 $\pm$ 30.2	310 $\pm$ 28.5 <sup>*</sup>	222.4 $\pm$ 29.8
Insulin+CAP+NaGc	350.7 $\pm$ 28	323 $\pm$ 45.6	273.1 $\pm$ 35.9 <sup>*</sup>	215.6 $\pm$ 39.9 <sup>*</sup>	144.4 $\pm$ 46.9 <sup>*</sup>
Control **	360 $\pm$ 15.2	366 $\pm$ 34.7	368.4 $\pm$ 43	349.3 $\pm$ 35.3	358.6 $\pm$ 37.3

<sup>\*</sup>  $p < 0.05$  compared with the control data at similar time interval as significant; <sup>\*\*</sup>Insulin in phosphate buffered saline solution (PBS) was used as control

As shown in Figure 1, blood glucose concentrations were decreased after administration of insulin into the duodenum, the first part of the gastrointestinal tract. Analysis of blood samples after 60 min of administration indicated a continuous antihyperglycemic effect of the formulations. In addition, the results showed an enhancing effect of enhancers especially NaGc after 45 min ( $p < 0.05$ ), on the formulations efficacy compared with formulations containing insulin alone.

As shown in Figure 2, anti-hyperglycemic effect of insulin continued in the jejunum, the second part of the gastro-intestinal tract but with less intensity. Mean while, no significant ( $p > 0.05$ ) enhancing effect between NaGc and NaSal was seen.

Figure 3 represents data related to the administration of three different formulations in rats' ileum, the third part of gastro-intestinal tract. As shown, there was no significant decrease in blood glucose ( $p > 0.05$ ) among the various formulations.

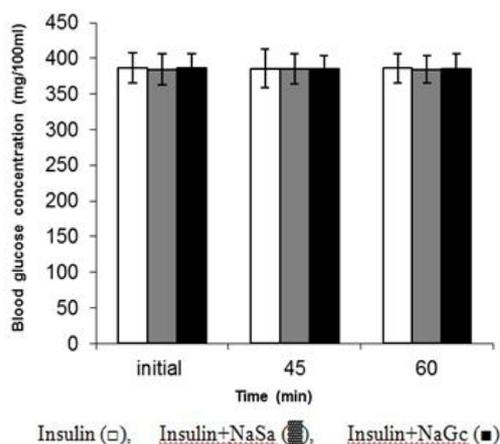
## DISCUSSION

The figures illustrate the glucose levels versus time profile following the administration of the insulin formulations coated with bile salts to male albino rats. In order to illustrate the protective capability of CAP in the insulin formulations coated with bile salts in stomach medium, the blood glucose concentrations (mg/100 ml) were determined. The in vivo stomach administration had indicated that presence of CAP in the insulin formulations coated with bile salts increase oral bioavailability of insulin in gastric medium. Regarding the results of the first part of the study, blood glucose levels remained constant during 120 min oral administration of formulations containing CAP into stomach and decreased dramatically after this period. Since the residence time of drugs in the stomach is about 2 h [16], it can be concluded that the

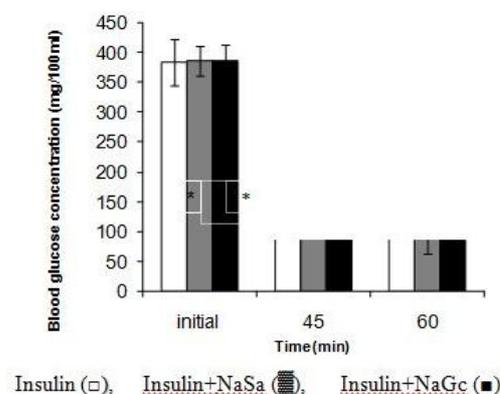
**Table 2:** Hypoglycemic effect following injection of different insulin formulations into rats duodenum, jejunum and ileum parts - relative to insulin (i.p.\*) (n = 5)

Site of injection	Formulation	Hypoglycemic effect (%)	
		45 min	60 min
Duodenum	Insulin	71	84
	Insulin+NaSal	85	90
	Insulin+NaGc	90	95
Jejunum	Insulin	34	54
	Insulin+NaSal	35	56
	Insulin+NaGc	39	60
Ileum	Insulin	9	5
	Insulin+NaSal	10	10
	Insulin+NaGc	5	8
	Insulin (IP)**	100	100

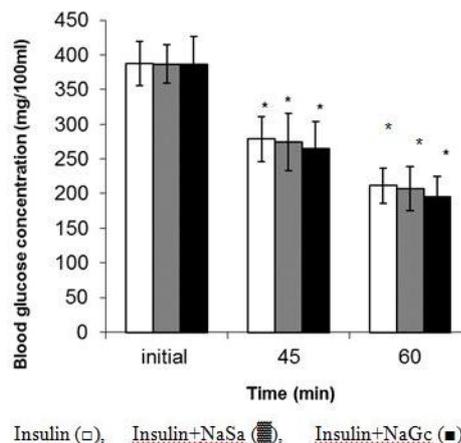
\*Intra-peritoneal administration as positive control; \*\* 100 % hypoglycemic effect via Intra-peritoneal administration at 45 and 60 min was 77.9 mg/100 ml and 70.5 mg/100 ml, respectively



**Figure 1:** The effects of injecting different insulin formulations into the rats stomach (n = 5, mean ± SEM);  $p < 0.05$  in comparison to negative control (normal saline intra-peritoneal) considered as significant



**Figure 2:** The effects of injecting different insulin formulations into the rats duodenum (n = 5, mean ± SEM);  $p < 0.05$  in comparison to negative control (normal saline intra-peritoneal) considered as significant;  $^{\Delta}p < 0.05$  in comparison to insulin formulation



**Figure 3:** The effect of injecting different insulin formulations into the rat's jejunum (n=5, mean ± SEM).  $^*p < 0.05$  in comparison to negative control (normal saline intra-peritoneal) considered as significant

formulations kept intact in this period, delivered to the first part of the small intestine in which CAP coating dissolved after 180 min following administration. It means that CAP is an effective coating for protecting insulin from chemical and enzymatic destruction during transit through stomach. A remarkable hypoglycemic effect was obtained following duodenal and jejunal injection of insulin with two bile salt enhancers NaGc and NaSal which could be due to increase in protein absorption in the presence of bile salt enhancers and is in accordance with the previous studies [5,17]. The results of Figures 1 and 2 indicated that NaSal and NaGC did not decrease blood sugar equally. Some investigators believed that NaGc has relatively low toxicity compared to other bile salts such as NaSal [9] with no side effect in human [18]. However, insulin suppositories containing 100 mg NaSal were showed safe and well tolerated by the volunteered individuals [19]. The other studies also suggested that high concentration of NaSal is needed to enhance the intestinal absorption of drugs [20]. As it has been shown in Figure 3, no decrease in blood glucose level after injection of insulin into ileum was seen. This demonstrates that insulin was not able to permeate from cell membrane of end parts of intestine even in presence of enhancers. As a result, it was shown that the two tested enhancer were site-dependent and their effectiveness was observed especially in the duodenum and with a little degree in jejunum which could be due to the site dependent of enhancers, although the reason was not fully understood.

The hypoglycemic effect after the administration of different formulations of insulin alone and coated insulin into rat stomach were shown in

Table 1. Importantly, the blood glucose level achieved the largest decrease (to  $144.4 \pm 46.9$  mg/100 ml) after oral administration of the Insulin+CAP+NaGc formulation with 10 IU/Kg insulin at after 180 min. Furthermore, the CAP shell protects insulin against proteolytic enzymes. It is likely that formulations containing cellulose acetate phthalate (CAP) protectors and bile salts enhancers may protect insulin during transit through the stomach. The percentage of hypoglycemic effect of injecting different insulin formulations into rat's duodenum, jejunum and ileum parts relative to insulin intra-peritoneal administration (100 % of hypoglycemic effect) at similar time interval were shown in Table 2. After direct administration, two bile salts formulations elicited a 90 % range degree (from 5 % to 95 %) of hypoglycemic effect of IP administration of insulin as positive control. Insulin-containing NaGc formulation showed more effective and sustained action over a period of 3 h. The results showed that formulations containing bile salts especially NaGc administrated into duodenum and with little degree in jejunum decreased blood glucose levels, compared to insulin intra-peritoneal formulations.

The mechanism of enhancing the absorption of drugs is still a subject of controversy. Several mechanisms have been proposed for mucosal absorption enhancement effect of enhancers such as micelle formation, solubilization, alteration of the mucus layer and reducing barrier integrity via the effect on tight junctions [7,15]. Additionally, NaGc is also found to have protease inhibitor effects and is considered as amino peptidase inhibitors. Accordingly, our findings suggest that co-administration of protease inhibitors with the enhancing ability and site dependent effect such as NaGc would be useful for improving the small intestine absorption of insulin.

## CONCLUSION

This study revealed that CAP can provide suitable protective effect against enzymatic degradation of insulin in the stomach. A hypoglycemic effect was observed following co-administration of insulin with bile salt enhancers NaGc and NaSal. Between two bile salt enhancers, NaGc had more significant enhancing effect on duodenal and a little extent, jejunal absorption of insulin. Finally, we can suggest that the effect of NaGc and NaSal on the intestinal absorption of insulin is site-dependent and the duodenum is thought to be the optimal site for insulin oral delivery.

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