

## The Enhancing Effect of Sodium Glycocholate and Sodium Salicylate on Rats Gastro-intestinal Permeability to Insulin

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### Abstract

There have been numerous efforts to formulate insulin into an oral dosage form. The major problems involved with the oral administration of insulin are acidic and enzymatic decomposition by the gastric medium, and poor absorption in the small intestine due to its macromolecular structure.

This study attempted to test the enhancing ability of two absorption enhancers, sodium glycocholate (Na-GC) and sodium salicylate (Na-Sal), in different parts of rat's gastro-intestinal tract. The amount of insulin in each formulation was 0.6 iu/kg body weight. The concentration of enhancers (Na-Sal or Na-GC) in each formulation was 10 µg/ml. Formulations made of insulin and enhancers were prepared and injected directly to stomach, duodenum, jejunum and ileum of anesthetized rats through an abdominal incision. Blood samples were taken at 45 and 60 min intervals. The glucose concentration was determined by the *o*-toluidine method. Injections (IP) of insulin and normal saline were positive and negative controls, respectively.

The blood glucose concentrations showed a significant decrease ( $p < 0.05$ ) due to the injection of insulin into duodenum, while the effect noted in jejunum was insignificant ( $p > 0.05$ ). Also, there was no anti-hyperglycemic effect accompanied by formulations administered into the stomach and ileum. It could be concluded that insulin, if formulated in a protected form to prevent acidic and enzymatic decomposition, in combination with such enhancers may overcome hyperglycemia due to insulin deficiency.

**Keywords:** Absorption enhancer; Salicylate; Glycocholate; Insulin.

### Introduction

In 1921 it was demonstrated that pancreas extract could decrease blood glucose concentration. In 1926, insulin was extracted and prepared as pure crystals. Insulin is a macromolecule which contains two polypeptide chains connected through two disulfide bonds. Its approximate molecular weight is 6 KDa.

Commercial preparations of insulin had formerly swine or bovine origin, although due to

the development of extraction and purification methods, now human insulin has been introduced and its commercial preparations have replaced animal insulin. Because of the peptide nature, if insulin administered orally, it would be denatured due to chemical and enzymatic constitution of GI tract. Hence, it can not be administered orally and injectable dosage forms are routinely in use. However, the injectable dosage forms present their own disadvantages. For example, they need high expenses for production and storage, and due to continuous consumption, they are potentially dangerous and

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painful for diabetic patients. Furthermore, there are always severe fluctuations in insulin blood concentrations after SC and IM injections, which could lead to insufficient therapy. Therefore, there have been extensive researches to formulate non-injectable preparations of insulin. In 1989, Chiou et al (1) proposed that among several penetration enhancers, saponin had the maximum effect to enhance insulin absorption through the eyes. Also Pillion et al (2) enhanced systemic absorption of insulin from nasal and ocular formulations, using a semi-synthetic quillaia saponin. By formulating into chitosan nanoparticles, nasal absorption of insulin was enhanced in a rabbit model (3).

Enhancers used in this study were sodium glycocholate (Na-GC) and sodium salicylate (Na-Sal). Their effectiveness to enhance mucosal absorption has been reported in several articles. Hersey et al (4) reported a 4-fold increase in permeability of rabbit mucosa to sucrose, when accompanied by bile salts as enhancer. In a study on rat's gastro-intestinal mucosa homogenate, Nakada et al (5) showed an enhancing effect of dihydroxy bile salts on calcitonin absorption. Sugiyama et al (6) evaluated the effect of some absorption enhancers, including Na-GC and Na-Sal, on rat's small and large intestine *in vitro*. They found a good correlation between *in situ* and *in vitro* result, using the Ussing chamber model. The effectiveness of Na-Sal to improve oral absorption of drugs is yet a subject of question (7). By evaluating of the effectiveness of Na-Sal in improving phenol red absorption in rat small intestine, no enhancing effect was observed. Using an intestine loop model and Na-Sal as enhancer, Zhang et al (8) showed a significant increase in leuprolide bioavailability in conscious rats.

The aim of the present study was to evaluate the effects of two widely studied penetration enhancers on gastro-intestinal absorption of insulin.

## Experimental

### Materials

The main materials used in this study were insulin (ActRapid Beef, Novo Nordisk), glucose

(Sigma, Germany), *o*-toluidine (Sigma, Germany), sodium glycocholate (Fluka, Germany) and sodium salicylate (Fluka, Germany)

### Methods

#### *Preparation of enhancer solutions*

5g of sodium glycocholate and sodium salicylate were individually dissolved in 100 ml of double distilled water. To assure their stability, the solutions were prepared just prior to the tests. The final enhancer concentration in each injection was one percent (*w/v*).

#### *Standard glucose solution*

200 mg glucose was added to 50 ml double distilled water. 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 *o*-toluidine method.

#### *Animal studies*

Male rats (Albino, mean weight 166 g, mean age 4 months) were fasted for 2 h 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.). Then 0.6 IU/kg insulin crystal in combination with an enhancer was injected into rats' stomach, duodenum, jejunum and ileum through a small abdominal incision. Using a syringe, blood samples were withdrawn from the ear vein at 45 and 60 min following administration. Samples were then centrifuged (Clements 2000 Centrifuge, Australia) and serum was separated. Insulin crystal injection and normal saline (I.P) were used as positive and negative controls, respectively.

#### *o-Toluidine reagent:*

The *o*-Toluidine method for glucose concentration measurement was used as described by Moorehead et al. Briefly, 3 g of reagent a grade thiourea was dissolved in 1 l of glacial acetic acid; then, 300 ml of *o*-toluidine was added and mixed for about 20 min. Next 700 ml of water was added and mixed again.

**Table 1-** The effect of injecting different insulin formulations into Rats' stomach, duodenum, jejunum and ileum, compared with the intra-peritoneal injection (I.P.) of insulin (n=s, mean±SEM)

Site of Injection	Formulation	Blood Glucose Concentration (g/100ml)		
		0 min	45 min	60 min
Stomach	Insulin	386.4±21.2	384.1±21.9	385.9±20.0
	Insulin+Na-Sal	385.5±20.5	384.9±21.0	385.6±18.8
	Insulin+Na-GC	385.9±21.1	384.2±19.1	385.7±20.1
Duodenum	Insulin	383.3±38.9	164.3±27	121.5±22.2
	Insulin+Na-Sal	386.1±25.7	121.0±18.8 *	99.5±36.5 *
	Insulin+Na-GC	386.9±26.1	106.5±19.9 *	85.5±29.1 *
Jejunum	Insulin	387.0±32.0	278.5±32.8	211.3±25.1
	Insulin+Na-Sal	386.8±27.5	274.5±41.1	207.5±31.8
	Insulin+Na-GC	386.2±40.1	264.8±39.5 *	195.6±29.4 *
Ileum	Insulin	385.4±23.3	354.1±27.7	365.2±25.3
	Insulin+Na-Sal	382.1±19.3	349.9±22.1	350.6±32.4
	Insulin+Na-GC	384.1±31.6	368.4±31.9	357.8±30.7
<b>Insulin (I.P.)</b>		<b>382.1±19.3</b>	<b>77.9±8.7</b>	<b>70.5±8.8</b>

\*p < 0.05 in comparison to negative control considered as significant

The final volume of this solution was adjusted to 1800 ml by the addition of distilled water (10).

#### Statistical method

The presented data are the mean of two experiments and 5 measurements. Results are shown as Mean±SEM. Student t-test was performed to compare test results with the control results samples. P<0.05 was assumed as significant difference.

### Results and discussion

The results obtained from administration of three different formulations into rats' gastro-intestinal tract are shown in table 1. Based on the results obtained, there is no significant (p>0.05) hypoglycemic effect due to insulin in the stomach. No difference could be seen among the three formulations within the gastric medium. In the second part of the gastro-intestinal tract, the duodenum, blood glucose concentrations show a significant fall (p<0.05). The presence of enhancers, especially sodium glycocholate, has potentiated the effect. This effect continues in jejunum, although the extent of decrease is much less than in duodenum.

Figure 1 shows no difference between the plasma glucose concentrations before and at 45 and 60 min intervals following the after administration of insulin; even when co-administered with the enhancers.

As shown in figure 2, blood glucose concentrations after administration of insulin in duodenum has significantly decreased (p<0.05). Analysis of blood samples after 60 min of

administration indicates a continuous anti-hyperglycemic effect of the drug. In addition, the results show a significant enhancing effect of Na-Sal, and especially Na-GC, on the drug efficacy (p<0.05).

Anti-hyperglycemic effect of insulin continues in jejunum, but with less intensity. Although, there is no significant (p>0.05) enhancing effect for Na-GC and Na-Sal. In all instances, the drug effect observed 60 min after administration was more than that at 45 min.

Figure 4 represents data related to the administration of three different formulations in rats' ileum. As shown, there was no significant decrease in blood glucose (p>0.05), and also no significant difference among the various formulations. Mean ± SEM value of blood glucose concentration for negative control in all experiments was 385.4 ± 25.1 mg/100 ml.

Previous studies have shown an increasing effect of bile salts and their derivatives on permeability of several mucosal membranes. NA-GC is a bile salt that improves permeability of biological membranes to many drugs. Its safety on mucocilliary clearance has been reported. On intranasal co-administration with insulin, Na-GC has shown less irritation than other bile salts. Also, it has been demonstrated that Na-GC is a relatively safe enhancer with no side effect in human(12).

The other compound used in the present study was sodium salicylate (NA-Sal), another molecule having both hydrophilic and hydrophobic properties. Several studies on Na-Sal have shown its ability to improve biological absorption. However, it has been discussed that

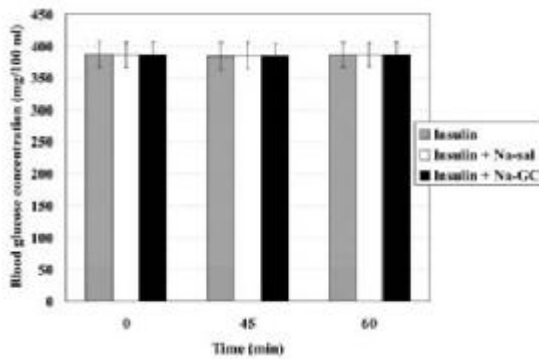


Figure 1. The effect of Injecting different insulin formulations into the Rats' stomach (n=5, mean±SEM)

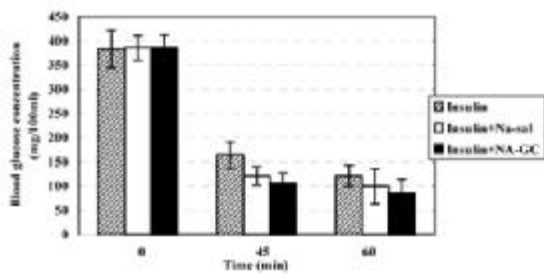


Figure 2. The effect of Injecting different insulin formulations into the rats' duodenum ( : p<0.05, n=5 and mean±SEM)

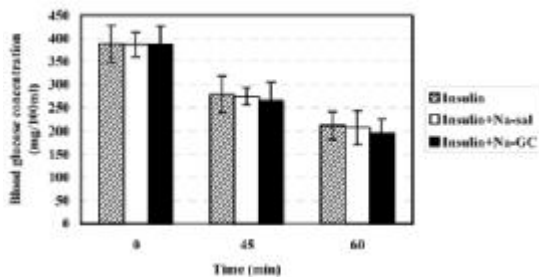


Figure 3. The effect of Injecting different insulin formulations into the Rats' jejunum ( : p<0.05, n=5 and mean±SEM)

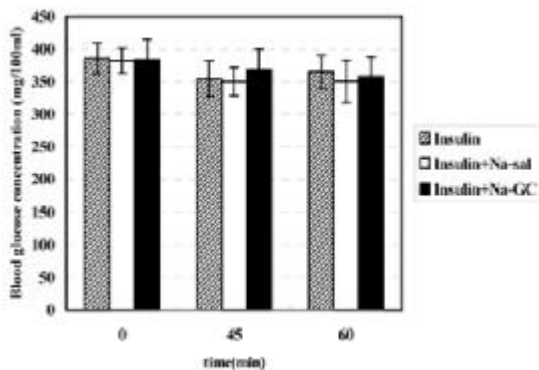


Figure 4. The effect of Injecting different insulin formulations into the rats' ileum (n=5, mean±SEM)

it may have less enhancing efficiency and more toxicity to biological membranes.

Our results show that, if placed in a proper absorptive site, Na-GC can improve insulin absorption. The appropriate pH of duodenum is a very important factor for both drug stability and enhancer activity. Therefore it could be suggested that at such a pH, Na-GC shows maximum surface activity, necessary for absorption enhancement activity. In addition to pH, permeation of hydrophilic compounds is affected by their molecular size and the size of intercellular tight junctions.

Due to the presence of Na-GC, absorption improvement might be seen in the other parts of rat intestine. However, there was no significant change ( $p>0.05$ ) in the blood glucose concentration following the injection of formulation into rats' stomach. Enzymatic and chemical decomposition of insulin in gastrointestinal fluid are the main reasons for this inefficiency.

The mechanism of enhancing the absorption of drugs is still a subject of controversy. The influence could be at the drug or mucosal membrane level (14). Several mechanisms have been proposed for mucosal absorption enhancement effect of enhancers, which contain both hydrophilic and hydrophobic chemical groups. Among them, micelle formation, solubilization, alteration of the mucus layer and a direct action on membrane are more accepted. According to the first theory, formation of reversed micelles within the mucous membrane may function as a large transient pore in the cell membrane through which drug could easily permeate (14). It has been reported that sodium tauro-dihydro-fusidate requires concentrations above its CMC to enhance the uptake of insulin in sheep (15).

Drug solubilization due to the presence of surfactant may be another possible mechanism for increasing permeability. However, it should only be applied for poorly soluble drugs.

The mucus layer covering the cellular surface of mucosa acts as a barrier to the diffusion of drug molecules. The third theory suggests that ionic surfactants are able to reduce mucus viscosity and elasticity, and a consequently the barrier function of the layer (14).

It is also possible that bile salts can open the tight junction between mucosal cells, making absorption of very large molecules (40000 MW) possible (14). According to the fourth theory, amphiphilic agents placed within the intercellular spaces and special sites, named tight junctions, in the mucosal membrane, decrease their interfacial tension, and thus increase the intercellular spaces. Therefore, drug molecules which can not permeate normally, will permeate the membrane easily. The mechanism, of course, explains the increase in absorption of water-soluble drugs; because intercellular fluids are aqueous in nature.

It has been shown that bile salt micelles have the ability to pass through the structure of mucus-producing cell cultures, suggesting that the enhancement action is due to the formation of calcium complexes with bile salts. Based on this theory, calcium depletion increases the permeability of intestinal epithelium (9). A similar mechanism has been suggested for the absorption enhancing effect of bile salts (14).

In conclusion, our results indicate the efficacy of Na-GC and Na-Sal to increase rats duodenum permeability to insulin. The enhancing effect of Na-sal was found to be less effective than that of Na-GC. Also, due to the hydrophilic nature of insulin and its relatively large molecular size, it seems that the direct effect of enhancers is the main mechanism of absorption enhancement action of Na-GC and Na-sal within the duodenum.

### Acknowledgements

This study was sponsored by Jondishapour University of Medical Sciences, Ahwaz. The contribution of Nokhbeh M. in animal experiments is also acknowledged.

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