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Nasal absorption enhancement of insulin by sodium deoxycholate in combination with cyclodextrins[1]

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KEY WORDS deoxycholic acid; cyclodextrins; insulin; absorption; nasal mucosa; leucyl aminopeptidase

ABSTRACT

AIM: To evaluate the nasal absorption enhancement of insulin by sodium deoxycholate (SDC) in combination with cyclodextrins (CD). **METHODS**: The concentration of glucose in blood was measured. Scanning electron microscopy technique was used to investigate the effect of enhancers on the nasal mucocilia. The effect of SDC in combination with CD on the leucine aminopeptidase (LAP) activity in the nasal mucosa was observed. **RESULTS**: Intranasal administration of insulin (4 U/kg) along with 0.75 % SDC/β-CD at a molar ratio of 1:2 slowly decreased the blood glucose levels of rats. The minimal blood glucose level was (72.6 ± 2.1) % of baseline, and which was lasted for 3 h. Though the decrement of blood glucose in 0.75 % SDC/β-CD (1:2) treated group was less than that in 0.75 % SDC treated group, there was no significant difference between the two groups in AOC_{0-t} values ($P > 0.05$). SDC (0.01 %) solution did not have any absorption prompting effect. Scanning electron microscopy investigation showed that 0.75 % SDC/β-CD (1:2) solution had no marked damage on rat nasal mucosa 4 h after nasal administration, while 0.01 % SDC still had some damage on the rat nasal mucosa. The inhibitory effect of SDC on the LAP activity was decreased from 89.2 % to 69.2 %, 71.5 %, 60.4 %, and 61.3 % in 0.75 % SDC/β-CD (1:1), 0.75 % SDC/β-CD (1:2), 0.75 % SDC/DM-β-CD (1:1), and 0.75 % SDC/DM-β-CD (1:2) treated group, respectively. **CONCLUSION**: Combining β-CD with SDC lowered the serious nasal ciliotoxicity of SDC and had a marked absorption promoting effect, which was not due to low concentration of SDC but was related with the inhibition of LAP activity.

INTRODUCTION

When peptide and protein drugs are administered with a variety of absorption enhancers, they can transport through the nasal membrane fast and effectively[1]. These enhancers work by one or a combination of mechanisms, which include a direct effect on the nasal mucosa; inhibitory effect on the proteolytic enzymes; opening of the tight junctions between cells[2]. Many *in vitro* or *in vivo* tests prove that most of these promoters have a damaging effect on the nasal membrane[3,4]. So the safety concern is raised for the use of such enhancers, especially for chronic therapies. In nasal administration, cyclodextrins (CD) can be used as absorption enhancers of peptide and protein drugs, which have low nasal bioavailability[5]. It is reported that CD could decrease the toxicity of some absorption enhancers including glycodeoxycholate, Laureth-9, and lysophosphatidylcholine[6,7]. And in our previous study, we found that the marked nasal ciliotoxicity of SDC, which is a potent nasal absorption enhancer, could be lowered in combination with β-CD or DM-β-CD at a molar ratio of 1:2.

A direct relationship has been found between the absorption enhancing effect of bile salts and acylcarnitines and the subsequent membrane damage[8,9]. We undertake this study to investigate whether combining sodium deoxycholate (SDC) with β-CD or DM-β-CD will influence the absorption enhancing effect of SDC or not.

MATERIALS AND METHODS

Drugs and reagents SDC was purchased from Sino-American Biotechnology Company. β-CD and DM-β-CD were obtained from Tokyo Food Institution,

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Japan. Insulin was purchased from Shanghai Biochemical Pharmaceutical Plant. Sodium pentobarbitone was obtained from Guangzhou Chemical Reagent Factory.

Formulation Insulin was dissolved in a phosphate buffer (pH 7.4). Before nasal administration, SDC (0.75 % or 0.01 %) combined with 4.1 % β-CD or 4.2 % DM-β-CD at a molar ratio of 1:2 was added into the solution. The various formulations were shown in Tab 1.

Tab 1. Formulations used in the nasal absorption studies.

Absorption enhancers	Insulin/ kU·L^{-1}	Molar ratio (SDC:cyclodextrin)
Control	24	-
0.75 % SDC	24	-
4.1 % β-CD	24	-
0.75 % SDC/4.1 % β-CD	24	1:2
4.2 % DM-β-CD	24	-
0.75 % SDC/4.2 % DM-β-CD	24	1:2
0.01 % SDC	24	-

Nasal absorption Nasal absorption studies were performed as described by Hirai *et al*[10] and modified by Fisher *et al*[11]. Briefly, male Sprague-Dawley (244 g ± 42 g, Experimental Animal Center, Fudan University, Grade Ⅱ, Certificate No 005) rats were fasted for 18 h with water *ad libitum*. They were anesthetized with an ip injection of sodium pentobarbitone at 60 mg/kg. The trachea was cannulated, and the oesophagus was tied to the tracheal cannula. The experiment was started 30 min after the operation. When necessary, anesthesia was maintained by further administration of sodium pentobarbitone at 15 mg/kg. The insulin solutions 4 U/kg were administered to rats by instilling into one side of the nasal cavity via microsyringes and a polyethylene tube (PE-50). Blood samples (150 μL) were collected from the caudal vein and the serum was isolated by centrifugation. The blood-glucose concentrations were determined within 30 min after sample collection by the glucose oxidase method using a reagent kit (Shanghai Shengfeng Biological Reagent Factory) and UV-visible spectrophotometer (Shanghai Third Analytical Instrument Plant). Blood glucose values were calculated as a percentage of the blood glucose concentration measured just before the start of the experiment. The area above the blood glucose versus time curves (AOC_{0-t}) was calculated using the trapezoidal rule. For statistical evaluation, an unpaired *t* test was used.

Scanning electron microscopy observations Rats were killed 4 h after the nasal administration of insulin solutions with 0.01 % SDC or 0.75 % SDC combined with β-CD at a molar ratio of 1:2 as enhancers. The nasal septum mucosa was removed, and its surface was washed with cold saline. Then the tissue sample was fixed with 2.5 % glutaraldehyde solution and 1 % osmic acid. The sample was dehydrated by a series of ethanol dilution, replaced by *n*-amyl acetate, dried at critical point of carbon dioxide, coated with gold by an ion coater, and examined under a scanning electron microscope (S-520, HITACHI Japan).

Determination of leucine aminopeptidase (μLAP) activity in the nasal mucose homogenate After the rats were decaptitated, the nasal septum mucosa was isolated and homogenized in a 10-fold volume of cold saline in a glass homogenizer. The homogenate was centrifuged at 9000 × *g* for 10 min at 4 ℃ and the supernatant was frozen till use. LAP activity of the nasal mucosa homogenate was estimated according to the modified Goldbarg method. Supernatant 50 μL was added to 0.5 mL of a substrate solution, prepared by dissolving *L*-leucylnaphtylamide hydrochloride 20 mg in 100 mL of phosphate buffer 0.05 mol/L (pH 7.0). The mixture was incubated at 37 ℃ for 5 min. After 1 mL of HCl 0.2 mol/L was added to terminate the enzymatic reaction, 1 mL of 4 % *p*-dimethylamino-benzaldehyde ethanol solution was added to the mixture. After 20 min, the optical density (*OD*) value of the mixture was measured at 450 nm.

RESULTS

Nasal absorption enhancement activity of SDC combined with β-CD Nasal administration of insulin (pH 7.4) to rats showed a slight fall in blood glucose (Fig 1). When insulin in combination with 4.1 % (w/v) β-CD was administered nasally to rats, the minimal blood glucose level and AOC_{0-t} value were markedly greater than those of control group. SDC was a powerful enhancer. When insulin solution was administered with 0.75 % SDC as an enhancer, the blood glucose reached the minimal level at about 45 min after administration. The minimal percentage of blood glucose in 0.75 % SDC group was lower and the AOC_{0-t} value was greater than that in the control and 4.1 % β-CD group. The minimal percentage of blood glucose

level in 0.75 % SDC/β-CD (1:2) group were higher than in 0.75 % SDC group ($P < 0.05$), but the difference of the AOC_{0-t} values between the two groups was not significant ($P > 0.05$) (Tab 2).

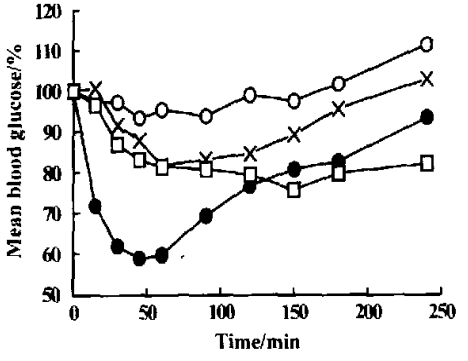

Fig 1. Changes in blood glucose concentration after nasal administration of insulin 4 U/kg with or without enhancer in rats. $n = 5$. (○) Control; (●) 0.75 % SDC; (×) 4.1 % β-CD; (□) 0.75 % SDC/β-CD (1:2).

Nasal absorption enhancement activity of SDC combined with DM-β-CD When insulin solution was administered with 4.2 % DM-β-CD, the decrease of blood glucose level and the increase of AOC_{0-t} values were greater than that in the control group. The blood glucose-time profile of 4.2 % DM-β-CD group was generally similar in shape with that of 0.75 % SDC group (Fig 2). In 0.75 % SDC/DM-β-CD (1:2) group, both the maximum fall in the blood glucose level and the AOC_{0-t} values were less than those in 4.2 % DM-β-CD group. The difference in minimal percentage of blood glucose between 0.75 % SDC/β-CD (1:2) and 0.75 % SDC/DM-β-CD (1:2) group was not significant (Tab 2).

Nasal absorption enhancement activity of 0.01 % SDC 0.01 % SDC had no marked effect on the absorption of insulin. There was no significant difference in the maximum decrease in blood glucose level, AOC_{0-t} values, and time course in 0.01 % SDC group compared with the control group (Tab 2, Fig 3).

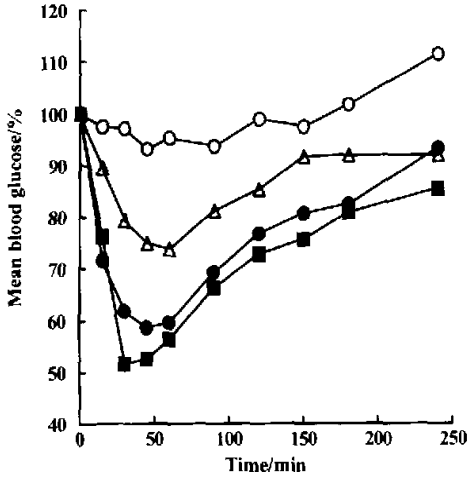

Fig 2. Changes in serum glucose concentration after nasal administration of insulin 4 U/kg with different enhancers in rats. $n = 5$. (○) Control; (●) 0.75 % SDC; (■) 4.2 % DM-β-CD; (△) 0.75 % SDC/DM-β-CD (1:2).

Scanning electron microscopy observation

As shown in Fig 4, cilia on the nasal mucosa treated with 0.75 % SDC solution combined with β-CD were similar to those in the negative control (physiological saline). However, the cilia on the nasal mucosa treated with 0.01 % SDC were partially denuded. The results suggested that 4 h after nasal administration of 0.75 % SDC combined with β-CD at molar ratio 1:2 had no

Tab 2. Hypoglycemic effect of nasal administration of insulin 4 U/kg with or without absorption enhancers in rats. $n = 5$. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control group. $^dP > 0.05$, $^eP < 0.05$ *vs* 0.75 % SDC group. $^gP > 0.05$ *vs* 4.1 % β-CD. $^kP < 0.05$ *vs* 4.2 % DM-β-CD group.

Absorption enhancers	Minimal percentage of blood glucose/%	T_{max}/min	AOC_{0-t}/% · min
Control	93 ± 6	42 ± 20	10 ± 8
0.75 % SDC	57 ± 18^b	39 ± 13	62 ± 32^b
4.1 % β-CD	79 ± 6^c	72 ± 34	23 ± 7^b
0.75 % SDC/ β-CD(1:2)	72.6 ± 2.1^{eg}	111 ± 58	42.2 ± 1.6^d
4.2 % DM-β-CD	48 ± 14^d	36 ± 8	69 ± 34^d
0.75 % SDC/DM-β-CD(1:2)	71 ± 15^k	66 ± 31	34 ± 13^k
0.01 % SDC	93 ± 3^a	60 ± 0	7 ± 6^a

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damage on the rat nasal mucosa, while SDC at low concentration of 0.01 % still had damaging effect on the nasal mucosa.

Inhibitory of LAP activity 0.75 % SDC caused more than 80 % inhibition of LAP activity, while the inhibition induced by β-CD and DM-β-CD was less than 30 % . Combining the two kinds of CD with SDC decreased the inhibitory effect of SDC (Tab 3).

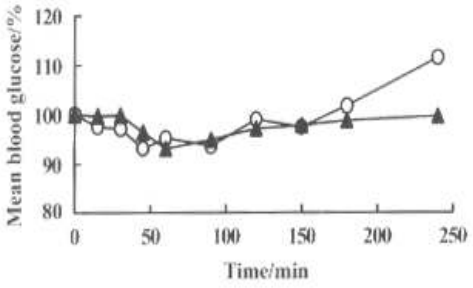

Fig 3. The hypoglycemic effect of intranasal administered insulin (4 U/kg) with or without 0.01 % SDC as absorption enhancer. $n = 5$. (○) Control; (▲) 0.01 % SDC.

Tab 3. Inhibitory effect of SDC with or without CD on the leucine aminopeptidase activity in the nasal mucosa. $n = 3$. $\bar{x} \pm s$. $^{c}P < 0.01$ *vs* 0.75 % SDC.

Absorption enhancers	Inhibition/%
2.1 %β-CD	23.5±3.5
4.1 %β-CD	26.9±2.5
2.1 % DM-β-CD	16.3±2.5
4.2 % DM-β-CD	29.5±1.6
0.75 % SDC/β-CD(1:1)	69.2±0.6[c]
0.75 % SDC/β-CD(1:2)	71.5±0.6[c]
0.75 % SDC/DM-β-CD(1:1)	60.4±1.0[c]
0.75 % SDC/DM-β-CD(1:2)	61.3±0.6[c]
0.75 % SDC	89.2±0.9

DISCUSSION

Nasal absorption studies showed that SDC and DM-β-CD were effective in promoting the intranasal absorption of insulin in rats, while β-CD was relatively less potent, which confirmed previous reports[12,13]. Administration of insulin with SDC combined with β-CD or DM-β-CD as absorption enhancer at molar ratio of 1:2 was less effective in lowering blood glucose levels compared with 0.75 % SDC alone. However it was interesting to note that the inhibitory effect of SDC/β-CD (1:2) on blood glucose level lasted for a longer time than

Fig 4. Scanning electron micrographs of the rat nasal mucosa 4 h after nasal administration of insulin solution 4 U/kg. A) 0.75 % SDC combined with β-CD at molar ratio of 1:2; B) 0.01 % SDC; C) Normal saline.

0.75 % SDC alone, so there was no significant difference in AOC_{0-t} values between the two groups. The scanning electron microscopy observations showed that 4 h after nasal administration of 0.75 % SDC/β-CD (1:2) did not damage the rat nasal mucosa, which was the same as chronic administration of it[14].

On the other hand, AOC_{0-t} value in 0.75 % SDC/DM-β-CD (1:2) group was less than that in 0.75 % SDC group. Based on these results we proposed that compared with DM-β-CD, β-CD was more suitable for lowering the ciliotoxicity of SDC as it could protect nasal mucosa against the damaging effect of SDC, while still maintaining partial enhancing ability.

It was difficult to interpret the results that in comparison with SDC alone, SDC in combination with β-CD produced a longer duration of insulin absorption. Gill *et al* reported that the presence of hydroxypropyl-β-cyclodextrin (HP-β-CD) did not reduce the absorption enhancing effect of Laureth 9, while it could lower its damaging effect on nasal mucosa[15]. They speculated that it might be due to the potent enhancing ability of Laureth 9 that it could promote the nasal absorption of insulin at low concentration. A balance between the extent of absorption enhancement and damaging effect, in other words, activity and safety, was maintained when Laureth 9 was used in combination with HP-β-CD. However the results of the present study did not support this. It was found that SDC at a low concentration of 0.01 % had no marked absorption enhancement activity but still had a damaging effect on the nasal mucosa.

It is well known that insulin is subjected to degradation by proteolytic enzymes such as LAP during passage through the mucosal membrane. The inhibitory effect of SDC/β-CD (1:2) or DM-β-CD on LAP activity may be one of possible mechanisms to explain the its absorption promoting effect.

In conclusion, combining SDC with β-CD at molar ratio of 1:2 was a convenient way to lower the nasal toxicity of SDC and still had an absorption promoting effect.

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脱氧胆酸钠-环糊精联用促进鼻对胰岛素的吸收[1]

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关键词 脱氧胆酸；环糊精类；胰岛素；吸收；鼻粘膜；亮氨酰氨肽酶

目的：评价β-环糊精(β-CD)或二甲基-β-环糊精与脱氧胆酸钠(SDC)联用对胰岛素的吸收促进作用. **方法**：通过胰岛素降血糖作用来反映其吸收效果；用扫描电镜观察单次给药后的鼻纤毛毒性；测定亮氨酰氨肽酶的活性. **结果**：0.75 % SDC/β-CD(1:2)联用时，鼻腔给予胰岛素产生的降血糖作用相对缓慢而持久，血糖最低值为初始的(72.6±2.1) %，血糖-时间曲线在1至4 h内较平稳. 虽然与0.75 % SDC组比较，两组的最低血糖水平有显著性差异，但血糖-时间曲线上面积 AOC_{0-t} 值无显著性差异($P>0.05$). 扫描电镜结果显示大鼠鼻腔给药4 h后粘膜纤毛无明显改变. 0.01 % SDC促吸收能力很弱，但仍有纤毛毒性. SDC与β-环糊精或二甲基-β-环糊精联用后，亮氨酰氨肽酶的活性抑制率由89.2 %下降至60 % - 70 %. **结论**：β-环糊精与SDC联用后，降低了SDC的纤毛毒性，但仍保留了较强的胰岛素吸收促进作用. 这种促进作用并不是由于SDC浓度的降低，而是与亮氨酰氨肽酶的活性被抑制有关.

（责任编辑 朱倩蓉）

全国第二届临床心脑血管病学术会议征文通知

由中国药理学会和中华医学会青岛分会联合上办的“全国第二届临床心脑血管病学术会议”定于2002年5月在昆明或西安召开，现将会议征文有关事项通知如下：

1、征文内容：心脑血管疾病的基础与应用基础研究；心脑血管疾病的临床研究；心脑血管药理的基础与应用基础研究；心脑血管疾病药物治疗学研究；心脑血管疾病介入治疗学研究；心脑血管疾病护理；临床研究方案的设计与统计方法研究等.

2、论文撰写要求：所投论文必须是未公开发表的学术论文，或具有本人研究工作的综述. 声明无一稿多投，文稿首页加盖单位公章，文责自负. 参照《中国临床药理学与治疗学》杂志论文格式要求撰写，中英文摘要按结构式书写. 来稿最好打印或通过E-mail投稿. 每篇论文需交审稿费30元.

3、论文截稿日期：2002年3月30日.

4、入选论文将以全文、摘要形式分期刊登于《中国临床药理学与治疗学》杂志(CN34-1206/R, ISSN 1009-2501)和《青岛医药卫生》杂志(CN37-1249/R, ISSN 1006-5571).

5、来稿请寄：安徽省芜湖市皖南医学院弋矶山医院内《中国临床药理学与治疗学》杂志编辑部收，邮政编码 241001，信封上请注明“心脑血管病学术会议”.

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