· 研究论文 ·

Deposition of insulin powders for inhalation *in vitro* and pharmacodynamic evaluation of absorption promoters in rats

YANG Dan-bo, ZHU Jia-bi*, ZHU Hui, ZHANG Xu-song

(*Institute of Pharmaceutics, China Pharmaceutical University, Nanjing 210009, China*)

Abstract: Aim To prepare insulin powder for inhalation by spray-drying technology, determine the deposition of the insulin powder formulation *in vitro* and preliminarily investigate hypoglycemic response of the dry powder with/without absorption promoters. **Methods** The depositions of the insulin powder for inhalation were determined by the China Pharmacopoeia 2000 version addenda XH and hypoglycemic effects were evaluated by testing serum glucose with glucose oxidase-peroxidase (GOD-PAP) method. **Results** The depositions of the spray-dried insulin powder for inhalation were more than 40% under various humidity and their changes were not significant when air flow was no less than 18 L·min^{-1}. The coadministration of insulin with 8 mmol·L^{-1}/dose sodium taurocholate [PA = 59.91%, C_{nadir} = (33 ± 6)%] and 10 mmol·L^{-1}/dose sodium deoxycholate [PA = 47.46%, C_{nadir} = (32 ± 7)%] induced a significantly greater decline in blood glucose levels, while coadministration with 1% sodium caprylate, 1% sodium dodecyl sulfate, 250 μg/dose lecithin, 10 mmol·L^{-1}/dose EDTA appeared to have no significant effect ($P > 0.05$). **Conclusion** Insulin powder for inhalation was relatively stable under various humidity conditions and different flow current. The use of 8 mmol·L^{-1}/dose sodium taurocholate and 10 mmol·L^{-1}/dose sodium deoxycholate could be able to potentially improve the bioavailability of insulin by pulmonary route.

Key words: insulin; pharmacodynamics; absorption promoter; powder for inhalation

CLC number: R943.4 **Document code:** A **Article ID:** 0513 - 4870(2005)12 - 1069 - 06

胰岛素吸入粉雾剂的体外沉降及大鼠体内吸收促进剂的药效学评价

杨丹波, 朱家壁*, 朱 慧, 张旭松

(中国药科大学 药剂研究所, 江苏 南京 210009)

摘要: 目的 采用喷雾干燥法制备胰岛素吸入粉雾剂, 对吸入粉雾剂体外沉降性质进行考察, 并对其吸收促进剂在大鼠体内的药效学进行初步研究。方法 采用中国药典(2000版)附录 XH的装置测定粉末的体外有效部位沉积量, 以葡糖氧化酶法(GOD-PAP法)测定大鼠的血糖浓度来评价其降血糖效果。结果 喷雾干燥法制得的胰岛素吸收粉雾剂沉积量在各湿度下均大于 40%, 在气流量 ≥18 L·min^{-1}的情况下其有效部位沉积量变化不大。8 mmol·L^{-1}/dose牛黄胆酸钠[PA = 59.91%, C_{nadir} = (33 ± 6)%]和 10 mmol·L^{-1}/dose去氧胆酸钠[PA = 47.46%, C_{nadir} = (32 ± 7)%]对胰岛素肺部吸收促进作用明显。而 1%辛酸钠、1%十二烷基硫酸钠、250 μg/dose卵磷脂和 10 mmol·L^{-1}/dose EDTA并未显示明显效果。结论 制得的胰岛素吸收粉雾剂沉积量受湿度影响小, 环境湿度依赖性和吸气流量依赖性小。8 mmol·L^{-1}/dose牛黄胆酸钠和 10 mmol·L^{-1}/dose去氧胆酸钠可有效促进胰岛素干粉吸入剂的降血糖效果。

关键词: 胰岛素; 药效学; 吸收促进剂; 吸入粉雾剂

Received date: 2005-02-21.

* Corresponding author Tel: 86 - 25 - 85338217, E-mail: zhujb@cpu.edu.cn

Introduction

The clinical efficiency of inhaled drugs depends on the site of deposition in the respiratory airway and the deposition pattern of the drug particles. The deposition of dry powders is influenced by patients' inhalation function[1], by the formulation of the powder as well as by the moisture. Therefore, we decided to establish an *in vitro* deposition as a relevant parameter that can be monitored rather easily for quality control purposes.

Although the pulmonary route has generated considerable interest as a valid, noninvasive, systemic route of drug administration, a major obstacle to the widespread use of pulmonary drug delivery is the relative impermeability of the lungs to many peptides/macromolecular drugs when they are administered without an absorption enhancer/promoter. The objective of this study was to investigate the pharmacodynamics of optimized formation in rats with/without potent and novel absorption promoters, including surface-active agents, sodium taurocholate, sodium deoxycholate, etc. These absorption promoters will affect the drug absorption by different mechanisms.

Materials and methods

Insulin and reagents Crystalline porcine insulin (28 u·mg^{-1}) was kindly supplied by Wanbang Co Ltd (Xuzhou, China). Citric acid-sodium citrate (Shanghai 1st Reagent Company, Shanghai), Acetonitrile (Hanbon Sci &Tech Company), Glucose-test reagent (Shanghai Rongsheng Biotech Co, Ltd); Mannitol (Shantou Xilong Chemical Co, Ltd). Samples were developed on our own. All other reagents were of analytical grade.

Assessment of spray-dried insulin powder deposition *in vitro* Powder containing insulin was prepared by the method previously described[2]. Insulin and various components were dissolved in redistilled water and filtrated. Then the solution was sprayed to obtain dry powder. Deposition *in vitro* was determined using a twin-stage impinger operated at a flow rate of 60 L·min^{-1}[3, 4] (China Pharmacopoeia 2000 version addenda XH, P 80). Fine particle dose[5] (FPD) was the amount of drug recovered from the lower stage of the impinger (H, stage-2) and the fraction of the delivered dose deposited in this stage is considered to be the respirable fraction; in the upper impingement chamber B, C (stage-0), the particles were cut-off as the powder intercepted in the throat and neck; chamber D was imitated to the particles inhaled into upper respiratory airway. 10 mL and 30 mL 0.9% physiological solution was presented in the upper and lower stages of the impinger, respectively. After operation, the solution was collected and the impinger stages were washed with further quantities of physiological saline. Finally, the solutions were bulked separately and determined by HPLC assay.

Pharmacodynamic studies in rats Male and female Sprague-Dawley rats with the weights of 180 - 220 g were obtained from the Experimental Animal Breeding Center (China Pharmaceutical University, Nanjing, China). Totally four groups with four female rats each group were randomly assigned to different treatments. Before administration of insulin in various doses with or without promoters, the rats were fasted overnight and anesthetized with sodium pentobartital (40 u·kg^{-1}, ip) in the experiments. After an animal had been secured on its back on an animal surgery board, the trachea was exposed. For intratracheal delivery of drugs, a microsyringe was inserted through the incision to depth of 12 - 15 mm according to the method of Enna and Schanker[6]. After the administration, the rats were catheterized with polyethylene tubing for breathing and monitored during the subsequent experiments. Venous blood samples (about 1 mL) were collected at each time point, centrifuged to obtain plasma for the analysis of glucose by the following procedure. The blood samples were allowed to clot for 15 min, centrifuged at 4 000 r·min^{-1} to 5 000 r·min^{-1} for about 1 min and separated out using a micropipette and transferred to siliconized 10 mL tubes. Serum glucose was determined by the glucose oxidase-peroxidase method[7].

The pharmacodynamics of insulin was studied after the administration of insulin in powder. The C_{nadir} (minimum serum glucose level) value alone, however, may not be a suitable parameter for the measurement of insulin pharmacodynamics since the basal level of blood glucose may vary between animals and experiments. Thus, we also employed the parameters of AAC (The Area Above Curve) and PA (Pharmacological Bioavailability). The area above the blood glucose-time curve and below the 100% line (AAC_{0-6h}) was estimated by the linear trapzoidal method[8] as following equation proposed by Ritschel WA and Ritschel GB[9]:

$$AAC = \sum_{i=0}^{n} \left[\left(\frac{C_0 - C_i}{C_0} \times 100 + \frac{C_0 - C_{i+1}}{C_0} \times 100 \right) \times (t_{i+1} - t_i) \right]$$

The relative pharmacological bioavailability (PA%) was calculated by comparing the AAC following by pulmonary administration with subcutaneous administration as following equation:

$$PA\% = AAC_{inhale} \times D_{sc} / (AAC_{SC} \times D_{inhale}) \times 100\%$$

Statistical datum analyses were carried out with Analysis of Variance and the Least-Squares Significant-Differences Multiple Comparison method at a significant level of 0.05.

Results and discussion

1 Deposition studies of spray-dried insulin from dry powder inhalers *in vitro*

1.1 The influence of pH value on deposition and solubility The equal isoelectric point of insulin is 5.3 and it will be separated out when the pH of the solution was in a range of 4.5 - 5.8. In addition, when the pH value is <2 or >10, the insulin will be degraded. Thus, we employed the hydrochloric acid and arginine to regulate the insulin solutions to pH values of 2, 4 and 7, separately, and the deposition and solubility rate were determined (Table 1). The results demonstrated that the dispersion quality was improved when pH value reached 4, however the solubility rate of the powder was decreased. On the other hand, the solubility rate was improved when the solution was more acidic while the dispersion was relatively worse. One of the possible reasons for such results might be due to the charges bearing under different pH values, for one of the adjuncts was hydrophobic amino acid whose equal isoelectric point was 6.3. The results showed pH 4 was the best condition for the optimized formulation and it was chosen for the further study.

Table 1 The influence of pH values on deposition and solubility rate of insulin powder for inhalation ($n=4$, $\bar{x} \pm s$)

pH	Stage-2 deposition/%	Solubility rate[a]
2.0	30.0 ±1.8	+ + +[c]
4.0	47.0 ±2.2	+ +[b]
7.0	40 ±4	+[d]

[a] Determined the time for solubility of equal quantity powders; [b] Indicates normal rate and as control group; [c] Indicates that the solubility rate is faster than that of the control group; [d] Indicates that the solubility rate is slower than that of the control group

1.2 The influence of environmental humidity on insulin powder deposition *in vitro* Drug Pulmonary Inhalers (DPIs) were dramatically subjected to the influence of the environmental humidity and also affected by whether the powder would be small enough to be delivered with accurate doses to patients[10]. The samples were stored for test objects at our laboratory under controlled conditions of various relative humidity at 45%, 54%, 67%, 74% and 82%.

The results obtained from the deposition in the successive stages of the twin impinger after storage at various relative humidity (RH) were showed graphically in Figure 1. After storage at various RHs, no significant decrease of the deposition at stage-2 of the twin impinger was observed, all of which were more than 40%. However, the stage-1 deposition increased slightly during storage under the condition. Thus, the deposition seems to be influenced by the different environmental humidity insignificantly.

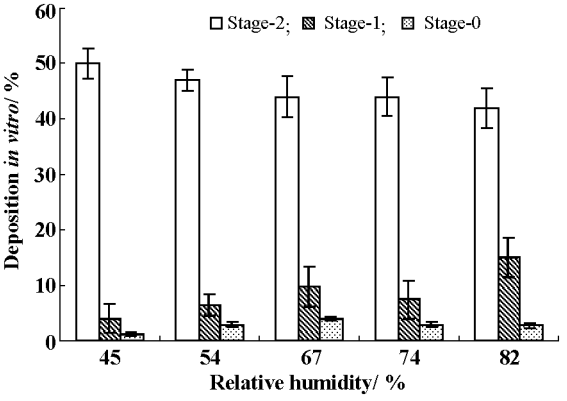

Figure 1 The influence of different environmental humidity on the deposition *in vitro* of insulin powder for inhalation ($n=4$, $\bar{x} \pm s$)

1.3 The influence of flow rate on the insulin powder deposition *in vitro* As DPIs system is driven by human autonomic respiration, the quantum of the respirable flow is possibly an important element influencing the distribution of the drug in lungs. Generally for an adult the air current is up to 60 L• min^{-1}, but for the respiratory patients and children, the air current is below 60 L• min^{-1}. Decrease in the respirable fraction was observed when the air flow was below 6 L• min^{-1}. However, when the air flow is above 18 L• min^{-1}, the dispositions are all above 40% as showed in Figure 2. Currently we still have no satisfactory explanation for this phenomenon, which will be investigated further in a follow-up study. The results for the depositions showed to be almost identical when flow reached 18 L• min^{-1} and would be relatively slightly influenced by insufficient flow.

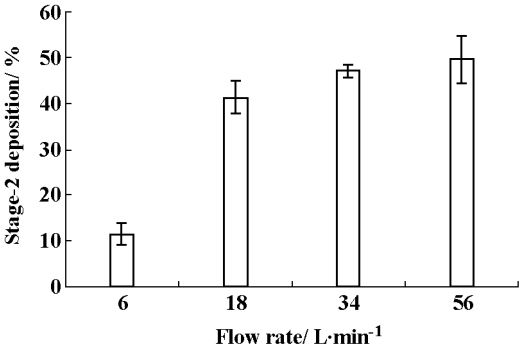

Figure 2 The influence of respiratory flow rate on the insulin powder deposition *in vitro* ($n=4$, $\bar{x}\pm s$)

1.4 The influence of spacer chamber on insulin powder deposition *in vitro* DPIs treatment is also limited by the inability of reliable cooperation from patients. Therefore, patients are often prescribed DPIs treatment from nebulizers or from pressurized metered-dose inhaler (pMDI)[11] and a spacer. A new concept recently devised the mechanical actuation of a DPI into a spacer for subsequent inhalation without need for active cooperation. This device provides a stable dried powder of fine particles without additives for accurate treatment[12]. Comparison between the deposition of with spacer (Figure 3) and without spacer showed significant difference for the changes in ratio of stage-2 to stage-1. The primary variables were from the decrease of the stage-1 deposition ($P<0.05$).

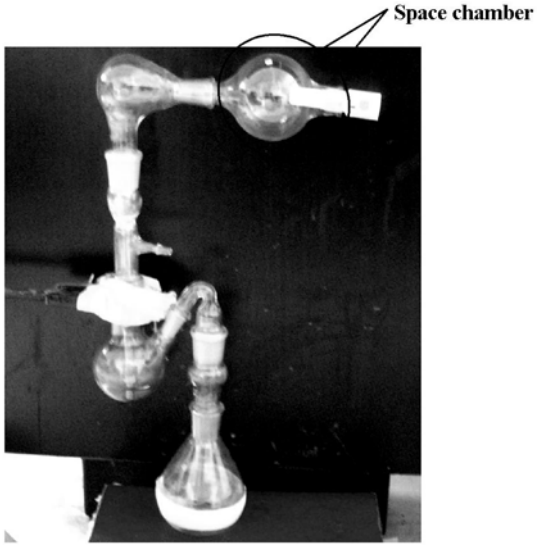

Figure 3 The apparatus of twin stage impinger with spacer chamber

As Table 2 showed, with spacer chamber, the fine particle cut-off increased nearly six times to the powder intercepted by upper respiratory tract, which may contribute to the decrease of tropical side-effect. However, even though the stage-1 deposition decreased significantly, the stage-2 deposition showed no significant difference. One of the possible reasons could be that the spacer intercepted part powders from being inhaled into the low deposition site.

Table 2 The influence of holding spacer on insulin powder deposition *in vitro* ($n=4$, $\bar{x}\pm s$)

Apparatus	Stage-2 deposition /%	Stage-1 deposition /%	Stage-2 /Stage-1
With spacer	26.0 ±2.0	2.8 ±1.3*	9.28*
Without spacer	24 ±5	16 ±12	1.52

Comparison between with and without space chamber, * $P<0.05$ *vs* without spacer

2 Pharmacodynamics in rats

The observed hypoglycemic effects following subcutaneous administration and intratracheal instillation was displayed in Figure 4. As to the calculation of pharmacological availability, the subcutaneous dose of 2.5 u·kg^{-1} has been utilized as the reference of insulin to rats. It was thought that the difference in AAC between the DPIs and S. C might be contributed by the characteristics of lung, large absorptive surface area (100 m^2), extensive vasculature, thin layer alveolar epithelium (0.01 - 0.02 nm) and short distance of air blood exchange passage. PA values were calculated and showed in Table 3 and demonstrated the relationship between the cumulative pharmacological response (AAC_{0-6h}) of insulin doses following subcutaneous administration and intratracheal delivery. The PA achieved 42.57% to that by S. C. Thus DPIs showed to be an effective alternative administration.

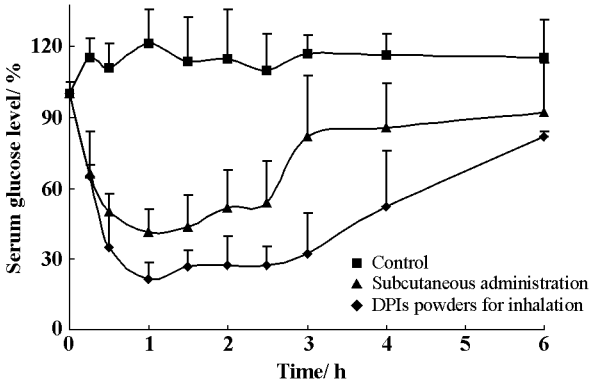

Figure 4 The blood glucose level of rat in three treatments of insulin ($n=4$, $\bar{x}\pm s$)

Table 3 Comparison of insulin hypoglycemic response by calculating AAC ($n = 4$, $\bar{x} \pm s$)

Formulation	AAC[a]	PA[b] /%	C_{nadir}[c] /%	T_{nadir}[d] /h
DPIs / 10 u• kg^{-1}	298.57	42.57	22 ±7	1
SC / 2.5 u• kg^{-1}	175.34	-	41 ±10	1

[a] The area above curve; [b] Pharmacological bioavailability; [c] The minimum serum glucose level; [d] The time when the serum glucose level was minimum

3 Preliminary evaluation of various absorption promoters in rats

The concentration-time profiles of blood glucose after pulmonary administration of insulin (2.5 u• kg^{-1}) were showed in Figure 5. In the presence of sodium cholate (sodium taurocholate and sodium deoxycholate) hypoglycemic responses were significant, as the PA% s were 59.91%, 47.46% and the C_{nadir} s were 33.00%, 32.03% (Table 4), respectively, in our present study. Sodium taurocholate showed significant difference from control group at the interval time of 1, and 1.5 hours point ($P < 0.05$), while sodium deoxycholate achieved significant difference at the point of 0.25, 0.5, 1 hour ($P < 0.05$). However, the toxicity of sodium cholate under high concentration needs further study. The mechanism of sodium taurocholate absorption enhancement is possibly by lowering transepithelial resistance and leads to an increased permeability of insulin across pulmonary cell monolayers[13]. While sodium caprylate, a salt of fatty acid, even though did not noticeably decreased the blood glucose level (PA = 19.23%), it extended for a longer period, T_{nadir} was 1.5 hours after the administration.

Since surfactants could increase the flexibility of biological membrane, it could enhance the transport of insulin across the epithelial membrane in the lung. Pulmonary inhalation insulin with Tween 80 had the PA of 43.30% and the C_{nadir} was 44.64%. Even though it is more effective than the control group, it failed to be significant difference ($P > 0.05$). Though similar to Tween 80, lecithin did not improve the pharmacological availability (35.47%), whereas the C_{nadir} was 49.07%, a bit more effective than the control group.

Table 4 Relative efficacy of various enhancers in promoting pulmonary insulin absorption

Absorption enhancer	AAC[a] /%	PA[b] /%	C_{nadir}[c] /%	T_{nadir}[d] /h
Sodium caprylate	74.94	19.23	57.0 ±1.2	1.5
Sodium taurocholate	233.50	59.91*	33 ±6*	1
Sodium deoxycholate	184.97	47.46*	32 ±7*	1
Sodium dodecyl sulfate	96.13	24.67	30 ±13*	0.5
Tween 80	168.75	43.30	45 ±16	1
EDTA	114.37	29.35	61 ±19	1.5
Lecithin	138.22	35.47	49 ±5	1
Control(without promoters)	165.91	42.57	54 ±11	1

[a] The area above curve; [b] Pharmacological bioavailability; [c] The minimum serum glucose level; [d] The time when the serum glucose level was minimum. * $P < 0.05$ *vs* control group

Another surfactant SDS improved the absorption of the pulmonary administration insulin well as the T_{nadir} was 0.5 h and the C_{nadir} was 29.78%, which is significantly different from that of the control group ($P < 0.05$). However, the PA (24.67%) did not show significant difference from that of the control group ($P > 0.05$) because the blood sugar has recovered

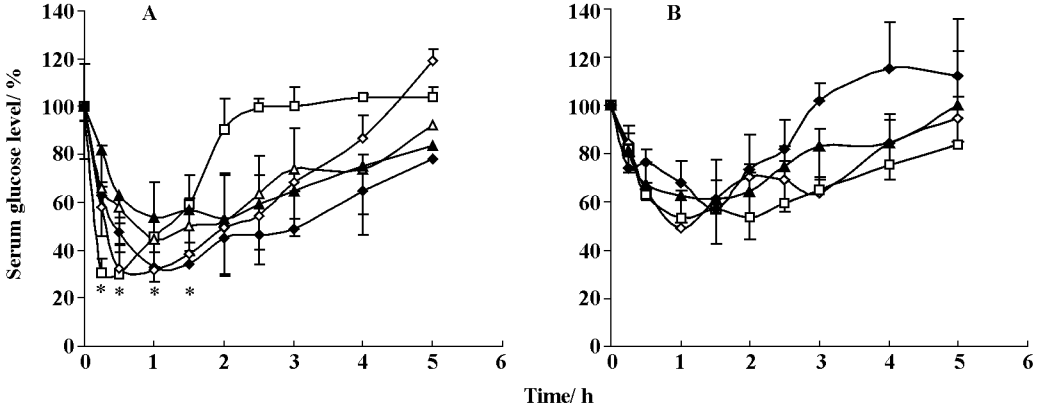

Figure 5 Concentration-time profiles of blood glucose of the insulin powders for inhalation in the presence of absorption enhancers ($n = 5$, $\bar{x} \pm s$); A: ▲—▲ Control; ◆—◆ Insulin with 8 mmol• L^{-1} /dose sodium taurocholate; △—△ Insulin with 1% Tween 80; □—□ Insulin with 1% sodium dodecyl sulfate; ◇—◇ Insulin with 10 mmol• L^{-1} /dose sodium deoxycholate. B: □—□ Control; ◇—◇ Insulin with 250 μg/dose lecithin; ▲—▲ 10 mmol• L^{-1} /dose EDTA; ◆—◆ Insulin with 1% sodium caprylate. * $P < 0.05$ *vs* control group

after 1.5 hours and reached the normal blood level at 2 hours.

The EDTA, one of chelate agents, was effective absorption enhancer used in nasal, enteric and rectal absorption of insulin[14] by affecting the permeability of tight junctions to increase paracellular transport[15]. However, in our present study, it gave no significant hypoglycemic response (PA = 29.35%, C_{nadir} = 61.24%, $P > 0.05$), which would be the result of the special structure of the mucous member in the lung by the mechanism of its inhibitory effect of drug hydrolysis or reduction of the viscosity of the lining fluid in the absorption site.

Acknowledgements: The authors acknowledge the financial supports from *Shenzhen Chuang Xinhui Bio Technologies Co., Ltd*, and National Key Pharmaceutics Laboratory of China Pharmaceutical University (Nanjing, China) for providing the experimental equipments and burdening some expense on relevant materials.

References

[1] Katz IM, Schroeter JD, Martonen TB. Factors affecting the deposition of aerosolized insulin [J]. *Diabetes Technol Ther*, 2001, **3**(3): 387 - 397.

[2] Zhu H, Zhu JB. Study on the powder properties of insulin dry powder inhalations [J]. *J China Pharm Univ* (中国药科大学学报), 2004, **35**(5): 424 - 428.

[3] Xiong LJ, Zhu JB. Preparation and pharmaceutical properties of salcatonin dry powder inhalations [J]. *Acta Pharm Sin* (药学学报), 2003, **38**(3): 218 - 222.

[4] Hiller FC, Mazumder MK, Wilson JD, *et al*. Aerodynamic size distribution, hygroscopicity and deposition estimation of beclomethasone dipropionate aerosol [J]. *J Pharm Pharmacol*, 1980, **32**(9): 605 - 609.

[5] Louey MD, Razia S, Stewart PJ. Influence of physicochemical carrier properties on the *in vitro* aerosol deposition from interactive mixtures [J]. *Int J Pharm*, 2003, **252**(1 - 2): 87 - 98.

[6] Enna SJ, Schanker LS. Absorption of saccharides and urea from the rat lung [J]. *Am J Physiol*, 1972, **222**(2): 409 - 414.

[7] Hyvarinen A, Nikkila EA. Specific determination of blood glucose with *o*-toluidine [J]. *Clin Chim Acta*, 1962, **7**: 140 - 143.

[8] Vaughan DP, Dennis M. Mathematical basis and generalization of the Loo-Riegelman method for the determination of *in vivo* drug absorption [J]. *J Pharmacokinet Biopharm*, 1980, **8**(1): 83 - 98.

[9] Ritschel WA, Ritschel GB. Rectal administration of insulin [J]. *Methods Find Exp Clin Pharmacol*, 1984, **6**(9): 513 - 529.

[10] Geuns, Eduard RM, Toren, *et al*. Decrease of the stage-2 deposition in the two inmpinger during storage of beclomethasone dipropionate dry powder inhalers in controlled and uncontrolled humidities [J]. *Eur J Pharm Biopharm*, 1997, **44**(2): 187 - 194.

[11] Drblik S, Lapierre G, Thivierge R, *et al*. Comparative efficacy of terbutaline sulphate delivered by Turbuhaler dry powder inhaler or pressurised metered dose inhaler with Nebuhaler spacer in children during an acute asthmatic episode [J]. *Arch Dis Child*, 2003, **88**(4): 319 - 323.

[12] Bisgaard H. Automatic actuation of a dry powder inhaler into a nonelectrostatic spacer [J]. *Am J Respir Crit Care Med*, 1998, **157**(2): 518 - 521.

[13] Fredrik J, Elisabeth H, Stefan E, *et al*. Mechanisms for absorption enhancement of inhaled insulin by sodium taurocholate [J]. *Eur J Pharm Sci*, 2002, **17**(1 - 2): 63 - 71.

[14] Katsuhiko O, Seigo I, Tsuguchika Y, *et al*. Intratracheal delivery of insulin absorption from solution and aerosol by rat lung [J]. *Int J Pharm*, 1992, **88**(1 - 3): 63 - 73.

[15] Hussain A, Arnold JJ, Khan MA, *et al*. Absorption enhancers in pulmonary protein delivery [J]. *J Control Release*, 2004, **94**(1): 15 - 24.