ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 May; 23 (5): 467-470
http://www.chinaphar.com

Hypoglycemic efficacy of pulmonary delivered insulin dry powder aerosol in rats[1]

CHEN Xi-Jing[2], ZHU Jia-Bi[3], WANG Guang-Ji, ZHOU Ming-Xia, XIN Fu-Meng, ZHANG Na, WANG Cheng-Xi, XU Yue-Ning (*Department of Drug Metabolism and Pharmacokinetics*; [3]*Zhongkun Institute of Pharmaceutics, China Pharmaceutical University, Nanjing 210009, China*)

KEY WORDS insulin; hypoglycemic agents; aerosols; biological availability

ABSTRACT

AIM: To observe the hypoglycemic efficacy of pulmonary delivery of insulin in dry powder aerosol form. **METHODS**: Insulin dry powder, made of insulin and other proper materials, was insufflated in rat lung from an incision in the throat. Meanwhile, insulin injection was administered to other rats. Glucose concentration in blood was determined in the following 7 h. The areas above the curve (AAC) of glucose concentration in blood were used to evaluate the efficacy. **RESULTS**: The percent minimum blood glucose levels, compared with the glucose levels before the administration, for pulmonary delivered insulin at the doses of 20, 10, 5, and 2.5 U/kg were 6.5 %, 16.6 %, 24.6 %, and 57.0 %, respectively. The AAC of insulin 5 U/kg by pulmonary delivery was very close to that of subcutaneous administration at the same dose. There was a linear relationship between AAC and the logarithmic dose of pulmonary delivered insulin. **CONCLUSION**: The pulmonary delivery of insulin acts effectively and rapidly.

INTRODUCTION

In recent years, pulmonary delivery of macromolecular proteins and peptides has attracted much attention, because enzymatic degradation of such agents restricts the gastrointestinal tract as a route of drug absorption into the systemic circulation[1-4]. Nearly all of such drugs need to be injected so far. It is aching and inconvenient for patients to use them. A large surface area, extensive vasculature, thin membrane, and low enzyme activity of the lung provide an ideal absorption environment for protein and peptide drugs.

Insulin, a compound with molecular weight of 5700, was considered to be the first polypeptide drug candidate to be marketed in this area. Inhaled insulin developed by some companies is now in phase Ⅲ clinical trial and will be delivered to market in recent years[5]. It is very important to make clear the dose-effect relationship and to compare the effects produced by aerosol and subcutaneous administration. Though there were many reports about the hypoglycemic effects produced by insulin following aerosol administration, there was much difference in those results[6,7]. It may due to the factors of formulas and methods to administrate the drug.

The present study was designed to evaluate the effects of insulin in dry powder aerosol in rats, and compare it to the effects produced by subcutaneous injection.

MATERIALS AND METHODS

Agents Insulin was produced by Xuzhou Wanbang Pharmaceutical Inc, China. Insulin dry powder aerosol was made of insulin and proper supplements by spray drying method (MD = 2.4 μm). The reagents for glucose assay were purchased from Shanghai Rongsheng Biotech Inc.

Rats Male Sprague-Dawley rats, $n = 72$, weighing 200-250 g, were fasted for 18-24 h prior to an experiment. Water was allowed *ad libitum*. Rats were divided into six groups (12 rats in each group). Four groups received insulin by pulmonary delivery (20, 10, 5, and 2.5 U/kg respectively), one group received 5 U/kg insulin by subcutaneous injection, and one group

[1] Project supported by Jiangsu New Drug Innovating Fund, No 9903022.
[2] Correspondence to Assoc Prof CHEN Xi-Jing.
Phn 86-25-327-1182. Fax 86-25-330-2651.
E-mail chenxj@jlonline.com
Received 2001-10-25 Accepted 2002-01-08

ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 May; 23 (5)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

received blank supplements of the dry powder aerosol as control.

Pulmonary and subcutaneous administration of insulin The rats were anesthetized by pentobarbital (45 mg/kg, ip). The rat's body temperature was maintained with 100 W light bulb in an air-conditioned room. The animal was rested on its back and the limbs were secured by taping them on the board. After the trachea was exposed, a microsyringe with silicon tubing (0.9 mm in diameter) was inserted through an incision made between the fifth and sixth tracheal rings caudal to the thyroid cartilage to a depth of 12 – 15 mm. We used a powder inhaler, according to Komada[4], to administer the insulin dry powder aerosol. For five groups, the amount of the powder (equaled to 0, 2.5, 5, 10, and 20 U/kg) was accurately weighed according to individual animal and filled into the capsules. After the insertion of the capsules to the inhaler, a needle was used to open the tow pores of the capsules. A compressed air stream, made by an injector, dispensed the content into aerosol and insufflated it into rat's lung *via* the tracheal incision. The other group received insulin 5 U/kg by sc injection. Blood samples (about 100 μL) were collected from the jugular vein at 0, 15, 30, 60, 90, 120, 150, 180, 240, 300, and 420 min. Serum was separated and 20 μL was used to assay the glucose by the glucose oxidase method[8].

Data analysis Blood glucose concentrations were normalized by subtracting the zero time glucose level. The area above the blood glucose-time curve (AAC) was calculated by the linear trapezoidal method as reported[5,6].

$$AAC_{0-n} = \sum_{i=0}^{n} \{ (C_0 - C_i)/C_0 \times 100\ \% + (C_0 - C_{i-1})/C_0 \times 100\ \% \} \times [t_n - t_{n-1}]/2$$

The percent minimum blood glucose level was calculated by $bG_{min}\% = (bG_{min}/bG_0) \times 100\ \%$, and t_{min} (the time to reach bG_{min}) was also chosen as a parameter for pharmacodynamic evaluation. The efficacy relative bioavailability was calculated by:
$F = AAC_{pulmonary}/AAC_{sc}$ at the same dose.

RESULTS

Both pulmonary and subcutaneous administration of insulin decreased the rats' blood glucose levels rapidly (Fig 1). The maximum efficacy reached at 1.0 – 2.0 h, and then, the blood glucose levels began to recover.

The correlation between the hypoglycemic response (AAC) and the pulmonary delivered insulin dose was shown in Fig 2. The Fig 3 represented the relation of AAC and the logarithmic dose of insulin.

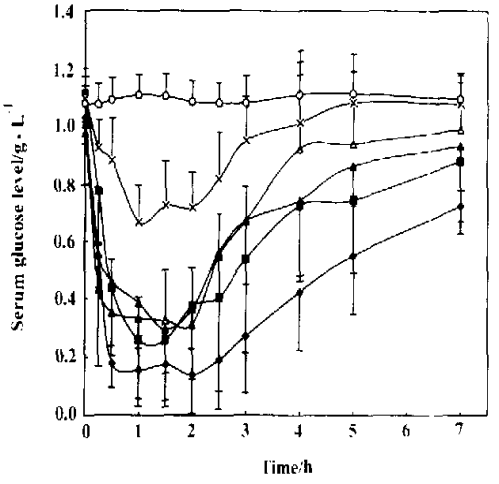

Fig 1. Profiles of blood glucose concentration following pulmonary administration of insulin 20 U/kg (◆), 10 U/kg (■), 5 U/kg (△), 2.5 U/kg (×), blank supplement (○) and sc injection of insulin 5 U/kg (▲). $n = 12$. $\bar{x} \pm s$.

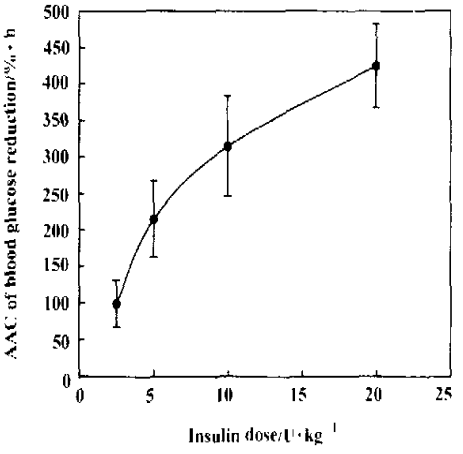

Fig 2. Dose-hypoglycemic efficacy curve after pulmonary delivery of insulin. $n = 12$. $\bar{x} \pm s$.

The percent minimum blood glucose level ($G_{min}\%$) for pulmonary delivered insulin at the doses of 20, 10, 5, and 2.5 U/kg could decrease the glucose level to 6.5 %, 16.6 %, 24.6 %, and 57.0 %, respectively compared to the glucose levels before the administration. The AAC of 5 U/kg of pulmonary delivery was very similar to that of subcutaneous administration at the same dose (Tab 1).

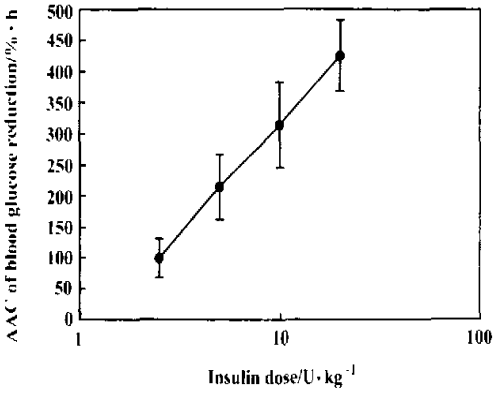

Fig 3. Logarithmic dose-hypoglycemic efficacy curve after pulmonary delivery of insulin. $n = 12$. $\bar{x} \pm s$.

Tab 1. Pharmacodynamic parameters related to the hypoglycemic effects of insulin administered pulmonarily and subcutaneously. $n = 12$. $\bar{x} \pm s$.

Route of administration	Dose /U·kg^{-1}	bG$_{min}$/ %	t$_{min}$/ h	AAC/ %·h	AAC ratio[1] / %
Pulmonary	20	6±4	1.6±0.6	425±57	
Pulmonary	10	17±8	1.4±0.5	315±69	
Pulmonary	5	25±14	1.2±0.8	215±53	99.95
Pulmonary	2.5	57±10	1.3±0.5	99±32	
sc	5	19±9	1.4±0.6	215±40	

[1] Ratio of AAC of pulmonary administration (insulin, 5 U/kg) to AAC of sc administration (insulin, 5 U/kg).

DISCUSSION

The efficacy of insulin delivered by pulmonary route was obvious from the dose of 2.5 to 20 U/kg in rats. The profile of blood glucose concentration by pulmonary administration was much similar with that by subcutaneous administration at the same dose of insulin 5 U/kg. The hypoglycemic effect was also very rapid, 15 min after pulmonary or subcutaneous administration, the blood glucose levels had been decreased evidently.

The relative bioavailability of pulmonarily delivered insulin was about 100 % compared to subcutaneous delivery. It indicated that the absorption proportion of insulin to the total dose from lung was the same as from subcutaneous position. This result was better than that reported earlier. Reasons besides the effects of dosage form (ie particle size and supplements), the method of administration may be one of the important factors. Authors of some articles used intratracheal instillation method by which the preparations must be dissolved in water or other solvents[6], so, they were not used in aerosol form and most of the drug cannot reach to the alveolus, which is an ideal place for peptides absorption.

The other factor influences bioavailability result is the calculating method. Because AAC was not in proportion with dose, we can only directly use AAC ratio to present bioavailability when the AAC values are very similar to each other, or else, it will not be proper and the results will differ from each doses.

It was also revealed that there was good linear relationship between the logarithmic doses and the efficacy parameter AAC in this experiment.

We concluded that the pulmonary delivery of insulin was effective and acting rapidly as well.

REFERENCES

1 Byron PR. Determinants of drug and polypeptide bioavailability from aerosols delivered to the lung. Adv Drug Deliv Rev 1990; 5: 107-32.
2 Robert F. Drug delivery takes a deep breath. Science 1997; 277: 1199-200.
3 Patton JS. Pulmonary delivery of drugs for bone disorders. Adv Drug Deliv Rev 2000; 42: 239-48.
4 Komada F, Iwakawa S, Yamamoto N, Sakakibara H, Ocumura K. Intratracheal delivery of peptide and protein agents: absorption from solution and dry powder by rat lung. J Pharm Sci 1994; 83: 863-6.
5 Inhaled insulin approaching market. Scrip 1888; 2456: 27.
6 Liu FY, Shao Z, Kildsig DO, Mitra AK. Pulmonary delivery of free and liposomal insulin. Pharm Res 1993; 10: 228-32.
7 Shen Z, Zhang Q, Wei S, Nagai T. Proteolytic enzymes as a limitation for pulmonary absorption of insulin: *in vitro* and *in vivo* investigations. Int J Pharm 1999; 121: 115-21
8 Ziv E, Kidron M, Raz I, Krausz M, Blatt Y, Rotman A, *et al*. Oral administration of insulin in solid form to nondiabetic and diabetic dogs. J Pharm Sci 1994; 83: 792-4
9 Tuoitou E, Rubinstein A. Targeted eternal delivery of insulin to rats. Int J Pharm 1986; 30: 95-9.

ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 May; 23 (5)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

胰岛素粉雾剂肺部给药对大鼠的降血糖作用[1]

R96 A

陈西敬[2]，朱家壁[3]，王广基，周明霞，辛芙萌，张　娜，王成琰，徐越宁（中国药科大学药代研究中心；[3]中昆药剂研究所，南京 210009，中国）

关键词　胰岛素；降血糖药；气雾剂；生物利用度

目的：观察胰岛素吸入粉雾剂肺部给药后的降血糖效果．**方法**：胰岛素与合适辅料制成的干粉经大鼠肺气管切口吹入肺中，测定随后 7 小时的血糖浓度，以血糖曲线上面积（AAC）为指标对其药效进行评价．**结果**：吸入胰岛素剂量为 20、10、5 和 2.5 U/kg 时，最低血糖浓度可分别降至给药前的 6.5 %，16.6 %，24.6 % 和 57.0 %；剂量为 5 U/kg 吸入给药的 AAC 值和 5 U/kg 皮下注射给药的 AAC 值相近；AAC 值与对数剂量间存在线性关系．**结论**：胰岛素肺部给药的降血糖效果明显且作用迅速．

（责任编辑　吴民淑）