新規バブルジェット式霧化装置を用いたインスリンの経気道的投与

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Original Article

Inhalative Administration of Insulin Using A New Bubble Jet Atomization Device

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Abstract Objectives : In this study, we attempted to perform inhalative administration of insulin using a new "bubble jet" atomization device based on ink jet printing technology and developed by Canon Inc. The aim of this study was to confirm the usefulness of the new device for achieving a hypoglycemic effect by insulin inhalation in normal rats.
Methods : Inhaled insulin (15 U/kg) or a control solution without insulin was administrated to each Wistar rat intratracheally using the bubble-jet atomization device. Blood glucose concentrations were measured at 0, 10, 20, 30, 60, 90 and 120 min after administration of insulin or control solution.
Results : The blood glucose concentrations in the inhaled insulin group were 63 ± 10 mg/dl (20 min), 43 ± 8 mg/dl (60 min) and 35 ± 9 mg/dl (120 min), while those in the control solution group were 80 ± 9 mg/dl ($p = 0.016$), 75 ± 10 mg/dl ($p < 0.001$) and 85 ± 27 mg/dl ($p < 0.001$). The blood glucose concentrations after administration of inhaled insulin were significantly lower than those after administration of control solution at all time points ($p < 0.05$) except 0 and 10 min.
Conclusions : We confirmed the hypoglycemic effect of inhaled insulin using the new bubble jet atomization device. These results proved that the new device could atomize insulin while maintaining its bioactivity.

Key words : Bubble jet technology, Inhaled insulin, New device, Pulmonary drug delivery

Introduction

The lungs are a very attractive target for the application of drugs and polypeptide hormones due to their large surface area (~140 m^2 in adult humans), low thickness of the alveolar epithelium (0.1 – 0.2 μm), high permeability, rich vascularization and minimal mucociliary clearance[1)]. Inhalative administration of drugs has been studied for the treatment of lung diseases, and numerous studies have demonstrated its efficacy as an optimal therapy for bronchial asthma and chronic obstructive pulmonary diseases. Recently, the pulmonary route has been the focus of attention as a novel drug delivery system for the treatment of systemic diseases, including diabetes mellitus.

Intensive insulin therapy has been shown to reduce long-term vascular complications in patients with type 1 and type 2 diabetes mellitus[2)3)]. A variety of injectable insulin products are available for the treatment of diabetes mellitus today[4)]. In addition, advances in the development of smaller and sharper needles and simple pen-injector devices have allowed better

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tolerability of percutaneous insulin administration. Nonetheless, quite a few patients object to the invasiveness of multiple daily injections. For this reason, alternative non-invasive modes of insulin administration, including nasal, oral-gastrointestinal, buccal, rectal, vaginal, dermal and of course pulmonary, have been tried[1)5)~7)]. To date, however, only the pulmonary route has proven effective. Recognition of the importance of aerosol dynamics in the 1990 led to significant progress in the delivery of inhaled insulin[1)], and several inhaled insulin products and corresponding inhalation systems are currently under investigation[8)~12)]. Inhaled insulin has an onset of action faster than subcutaneously injected insulin and provides more physiological response to a meal. Inhaled insulin is therefore best suited for the coverage of single meals, but not for basal or overnight administration. All pulmonary insulin delivery systems under investigation are used to deliver regular insulin, either in powder form or in solution.

In this study, we attempted the inhalative administration of insulin using a new "bubble jet" atomization device developed by Canon Inc. Bubble jet technology is an ink jet printing technology that makes use of the high pressure of vapor bubbles generated by film boiling of liquid inks[13)]. We applied this technology to our new inhalation device. A bubble jet print head consists of a series of ejection chambers built into micro-heaters. Film boiling is completely different from nucleate—or "normal" —boiling. In film boiling, liquid inks contact the heater surface, which is kept at a very high temperature (about 300 degrees Celsius), and vaporize rapidly. As a result, ink droplets are ejected from the chamber nozzle by the high pressure induced by vapor bubbles (Fig. 1). Because the vapor bubbles insulate the liquid inks from the heaters, the liquid inks are blocked from the heat source. We considered that, in theory, it would be possible to atomize insulin using the bubble jet technology. By changing the nozzle size, it would be possible to control particles of a variety of sizes, ranging from 1 – 20 μm. Moreover, by using a built-in IC chip, the system would be able to precisely control the amount of atomization and maintain a record of the history of use.

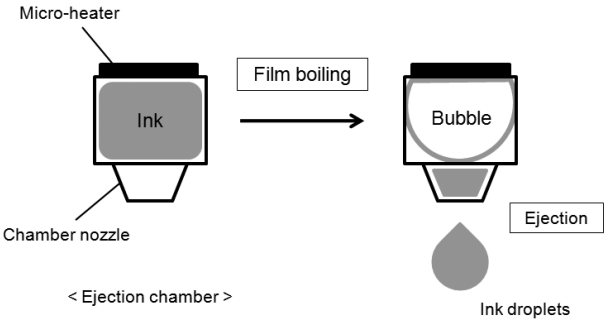


Fig. 1 Principles of the bubble jet technology. Liquid inks in an ejection chamber contact the surface of a micro-heater and are rapidly vaporized by film boiling. The high pressure produced by the vapor bubbles causes ink droplets to be ejected from the chamber nozzle.

This was the first in vivo study using such a bubble jet atomization device. The aim of this study was to confirm the usefulness of the new device for inducing a hypoglycemic effect via insulin inhalation in normal rats.

Materials and Methods

Ten healthy male Wistar rats (11 weeks old) were purchased from Japan SLC, Inc. (Shizuoka, Japan). They were fasted overnight but allowed free access to water before the study. The rats' body weights ranged from 248 to 265g, and they were divided into two groups. Five rats were assigned to receive inhalation of 15 U/kg regular human insulin (novo Nordisk Pharma, Tokyo, Japan), and another five rats to receive inhalation of a control solution without insulin. The animals were anesthetized with an intraperitoneal injection of 50 mg/kg sodium pentobarbital. After a 14-gauge catheter (Terumo, Tokyo, Japan) was inserted into each trachea under anesthesia, the animals were ventilated with a respirator (Model 683 rodent ventilator; Harvard Apparatus, South Natick, MA). Subsequently, insulin or control solution was administrated to the animals using

the bubble jet atomization device for 10 min (Fig. 2). The conditions of the mechanical ventilation were as follows : tidal volume 1.6 – 1.7 ml, respiration rate 60/min. Blood samples were collected from the caudal vein before administration (0 min), and at 10, 20, 30, 60, 90 and 120 min after administration of insulin or control solution. Blood glucose concentrations were measured by a blood glucose analyzer (Glucocard Diameter α; Sanofi-aventis K.K., Tokyo, Japan). The particle size of atomized insulin via intubation catheter was measured by a laser diffraction particle size system (Spraytec; Malvern Instruments, Ltd., UK).

All rats were sacrificed after the last blood sampling (at 120 min), and both lung tissues were fixed in 10% buffered formalin. After embedding in paraffin, the sections were prepared and stained with hematoxylin and eosin.

The experiments were carried out under both the Guidelines for Animal Experiments of Kyushu University, and the Law and Notification of the Japanese government.

Statistical analysis

Blood glucose concentrations were analyzed by two-way repeated-measures ANOVA and Tukey test. P-values < 0.05 were considered to be significant.

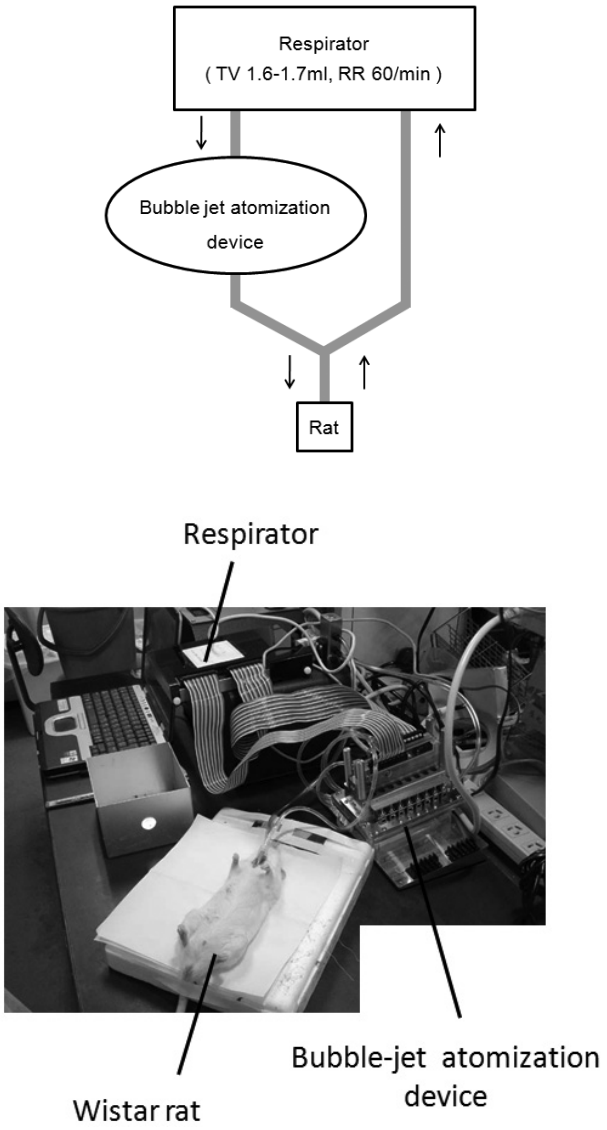


Fig. 2 Scheme of the study. The rats were connected to a respirator (Model 683 rodent ventilator ; Harvard Apparatus), and administrated insulin or control solution by the bubble jet atomization device for 10 min.

Results

Hypoglycemic effect. As shown in Figure 3, in response to administration of inhaled insulin (15 U/kg), blood glucose levels began to decrease at 10 min. The blood glucose concentrations in the inhaled insulin group were 92 ± 13 mg/dl (0 min), 63 ± 10 mg/dl (20 min), 43 ± 8 mg/dl (60 min) and 35 ± 9 mg/dl (120 min), while those in the control solution group were 90 ± 17 mg/dl (0 min, not

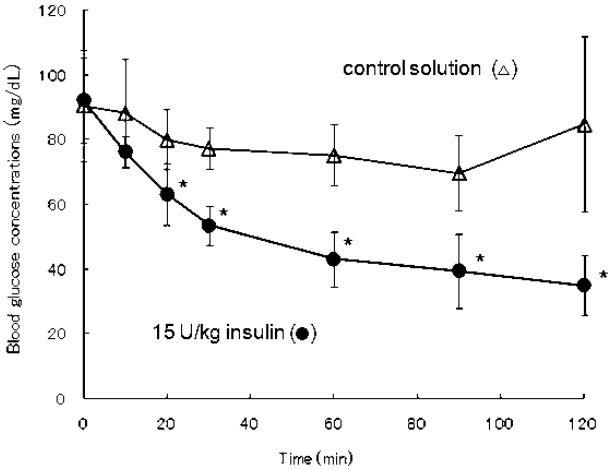
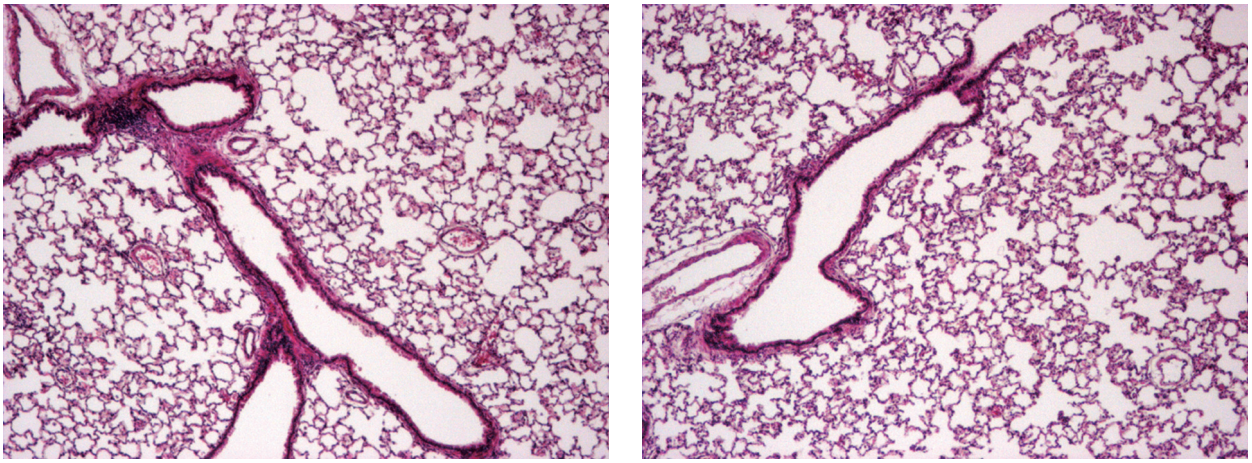


Fig. 3 Blood samples were collected from the caudal vein before administration (0 min), and at 10, 20, 30, 60, 90 and 120 min after administration. The blood glucose concentrations after administration of inhaled insulin (15 U/kg) were significantly lower than after administration of control solution at all time points except 0 and 10 min. * $p < 0.05$



(a) (b)

Fig. 4 Histological findings of the rat lung 120 min after administration of inhaled insulin (a) or control solution (b). Acute inflammatory changes were not observed in the bronchial walls or lung parenchyma. (Original magnification : × 40)

significant), 80 ± 9 mg/dl (20 min, $p = 0.016$), 75 ± 10 mg/dl (60 min, $p < 0.001$) and 85 ± 27 mg/dl (120 min, $p < 0.001$). In short, a significant difference in blood glucose reduction between the two groups was observed at all time points ($p < 0.05$) except 0 min ($p = 0.825$) and 10 min ($p = 0.072$).

Particle size. The particle size of atomized insulin was 3.8 – 4.9 μm.

Histology. Figure 4 shows the histological findings of the rat lungs at 120 min after administration of inhaled insulin (Fig. 4a) or control solution (Fig. 4b). No acute changes such as inflammation or edema were observed in the bronchial walls and lung parenchyma in the rats receiving insulin inhalation.

Discussion

As shown in Figure 3, we confirmed the usefulness of the new bubble jet atomization device for inducing a hypoglycemic effect via inhaled insulin in normal rats. This result suggests that inhaled insulin atomized by this new device was absorbed into the systemic circulation after administration. It was proved that the new device could atomize insulin while maintaining its bioactivity. The generation of high pressures of vapor bubbles by film boiling is an essential characteristic of the bubble jet technology. Thus it is a very important question whether or not the bioactivities of the atomized agents are maintained, since the heat treatment method tends to denature the agents, particularly in the case of proteins and peptides. In the case of insulin, this study showed that the bubble jet atomization device had no significant influence on its bioactivity. There are two possible reasons for this finding : 1) the required time for heat treatment was extremely short—on the order of microseconds ; 2) during the process of the film boiling, the vapor bubbles insulated the insulin particles from the heat source.

The size of the atomized insulin particles was 3.8 – 4.9 μm. Although the same measuring device was not used, the particle size in this study was comparable to that of other pulmonary insulin delivery systems. AERx iDMS system (Aradigm Corporation and Novo Nordisk) generates a liquid insulin aerosol of 2 – 3 μm diameter particles[9)14)]. AFRESA™ (Mannkind Corporation) delivers a dry powder formulation with 2 – 5μm diameter particles[15)]. The optimal size for delivery to the alveoli is 1 – 3 μm in aerodynamic diameter[16)~18)]. Larger particles (> 5 μm) are deposited predominantly in the oropharyngeal regions and upper airways, whereas much smaller particles (< 1 μm) are mostly exhaled. In general, 1 – 5 μm diameter particles are deposited

in the small airways and alveoli, and more than 50% of the 3 μm diameter particles are deposited in the alveolar region. Particles less than 3 μm in diameter have an approximately 80% chance of reaching to the lower airways, and 50 – 60% are deposited in the alveoli[19]. So, improving the device in order to control the particle size to below 3 μm may enable more efficient delivery of inhaled insulin.

Since the bubble jet atomization device can generate a variety of particle sizes in the range of 1 – 20 μm by changing the nozzle size, the device may be useful to deliver agents efficiently to a particular target area, such as the upper airway, the bronchial trees or the alveolar regions. In addition, it is a new and unique characteristic of the device that an IC chip is built in, which enables precise control of the amount of atomization and a recorded history of use. Most delivery systems for inhaled insulin under investigation are limited with respect to the inhalation dose. However, because the present system allows digital control of the amount of atomized agents, it could allow the supply of an optimal dose to each patient. In addition, the recording of inhalation data would make detailed information available for physicians and co-medicals, which could lead to better disease control in individuals.

There are two differences between the conditions used in this study and those used for physiological drug inhalation. In this study, the insulin was administered through the tracheotomy orifice. This means that the insulin reached to the lower bronchus without passing the oral cavity, oropharyngeal regions or upper airways. The other difference is that the drug was pushed into the airway under positive pressure. This could have changed the efficiency of inhaled insulin delivery compared with voluntary inhalation, because the aerosol velocity and inspiratory flow rate are the major determinants of aerosol delivery to the lungs[20].

As shown in Figure 4, no acute morphological change was observed in the bronchial walls and lung parenchyma. Since the histological analysis was performed after a single inhalation, more studies are required to evaluate the safety after repeated inhalations. Some adverse effects of inhaled insulin have been reported in clinical trials. They include cough, upper-respiratory-tract infections, and changes in pulmonary functions[21]. In clinical trials of Exubera® (Nektar Therapeutics and Pfizer) which was the first inhaled insulin to receive FDA approval in 2006 and withdrawn from the market only two years later, the incidence of new primary lung cancer per 100 patient-years of exposure was 0.13 (five cases over 3800 patient-years) for Exubera® and 0. 03 (one case over 3800 patient-years) for comparators[4]. Because these patients diagnosed with lung cancer had a prior history of cigarette smoking, association with inhaled insulin was regarded as inconclusive. Insulin is thought to be a weak growth factor under some experimental conditions, and is known to bind to the insulin-like growth factor-1 (IGF1) receptor with a potency < 1/100 that of IGF1[17]. The long-term safety of inhaled insulin is still not clear.

This was the first *in vivo* study using the bubble jet atomization device. Administration of inhaled insulin using this new device significantly decreased blood glucose concentrations compared to the administration of control solution. This proved that the new device could atomize insulin while maintaining its bioactivity. Serum insulin and C-peptide levels were not assessed in this study, so pharmacokinetic (PK) and pharmacodynamic (PD) studies must be performed as a next step.

The pulmonary route is currently being studied as a potential drug delivery system for not only insulin but also other pharmacological agents, e.g., vaccines, anticancer agents and opioids[22)~24]. The development of more efficient delivery devices will likely expand the potential for such inhalation therapy.

Conclusions

This was the first in vivo study using a new bubble jet atomization device based on ink jet printing technology and developed by Canon Inc. We confirmed the hypoglycemic effect of inhaled insulin using the new device. These results proved that the new device could atomize insulin while maintaining its bioactivity.

Acknowledgments

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References

1) Owens DR : New horizons-alternative routes for insulin therapy. Nat Rev Drug Discov. 1 : 529-545, 2002.
2) The Diabetes Control and Complications Trial Research Group : The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Eng J Med. 329 : 977-986, 1993.
3) UK Prospective Diabetes Study (UKPDS) Group : Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes. Lancet. 352 : 837-853, 1998.
4) Roach P : New insulin analogues and routes of delivery : pharmacodynamic and clinical considerations. Clin Pharmacokinet. 47 : 595-610, 2008.
5) Cefalu WT : Evolving strategies for insulin delivery and therapy. Drugs. 64 : 1149-1161, 2004.
6) Shaikh IM, Jadhav KR, Ganga S, Kadam VJ and Pisal SS : Advanced approaches in insulin delivery. Curr Pharm Biotechnol. 6 : 387-395, 2005.
7) Heinemann L, Pfutzner A and Heise T : Alternative routes of administration as an approach to improve insulin therapy : update on dermal, oral, nasal and pulmonary insulin delivery. Curr Pharm Des. 7 : 1327-1351, 2001.
8) Fuso L, Pitocco D and Incalzi RA : Inhaled insulin and the lung. Curr Med Chem. 14 : 1335-1347, 2007.
9) Schuster J, Rubsamen R, Lloyd P and Lloyd J : The AREX aerosol delivery system. Pharm Res. 14 : 354-357, 1997.
10) Ghosh S and Collier A : Inhaled insulins. Postgrad Med J. 83 : 178-181, 2007.
11) De Galan BE, Simsek S, Tack CJ and Heine RJ : Efficacy and safety of inhaled insulin in the treatment of diabetes mellitus. Neth J Med. 64 : 319-325, 2006.
12) Skyler JS, Cefalu WT, Kourides IA, Landschulz WH, Balagtas CC, Cheng SL and Gelfand RA : Efficacy of inhaled human insulin in type 1 diabetes mellitus : a randomized proof-of-concept study. Lancet. 357 : 331-335, 2001.
13) Asai A : Development of bubble jet printers. Nagare. 24 : 603-608, 2005.
14) Henry RR, Mudaliar SR, Howland WC 3rd, Chu N, Kim D, An B and Reinhardt RR : Inhaled insulin using the AERx Insulin Diabetes Management System in healthy and asthmatic subjects. Diabetes Care. 26 : 764-769, 2003.
15) Rave K, Potocka E, Heinemann L, Heise T, Boss AH, Marino M, Costello D and Chen R : Pharmacokinetics and linear exposure of AFRESA^{TM} compared with the subcutaneous injection of regular human insulin. Diabetes Obes Metab. 11 : 715-720, 2009.
16) Byron PR : Prediction of drug residence times in regions of the human respiratory tract following aerosol inhalation. J Pharm Sci. 75 : 433-439, 1986.
17) Scheuch G and Siekmeier R : Novel approach to enhance pulmonary delivery of proteins and peptides. J Physiol Pharmacol. 58 Suppl : 615-625, 2007.
18) Patton JS, Bukar JG and Eldon MA : Clinical pharmacokinetics and pharmacodynamics of inhaled insulin. Clin Pharmacokinet. 43 : 781-801, 2004.
19) Labiris NR and Dolovich MB : Pulmonary drug delivery. Part 1 : Physiological factors affecting therapeutic effectiveness of aerosolized medications. Br J Clin Pharmacol. 56 : 588-599, 2003.
20) Laube BL : Treating diabetes with aerosolized insulin. Chest. 120 : 99S-106S, 2001.
21) Mastrandrea LD : Inhaled insulin : overview of a novel route of insulin administration. Vasc Health Risk Manag. 6 : 47-58, 2010.
22) Low N, Kraemer S, Schneider M and Restrepo AM : Immunogenicity and safety of aerosolized measles vaccine : Systematic review and meta-analysis. Vaccine. 26 : 383-398, 2008.

23) Sharma S, White D, Imondi AR, Placke ME, Vail DM and Kris MG : Development of inhalational agents for oncologic use. J Clin Oncol. 19 : 1839–1847, 2001.
24) Overhoff KA, Clayborough R and Crowley M : Review of the TAIFUN multidose dry powder inhaler technology. Drug Dev Ind Pharm. 34 : 960–965, 2008.

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（和文抄録）

新規バブルジェット式霧化装置を用いたインスリンの経気道的投与

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【目的】本実験で我々は，キヤノン株式会社がインクジェットプリンター技術を応用して開発した，新規バブルジェット式霧化装置を用いてインスリンの経気道的投与を試みた．本実験の目的は，この新規霧化装置を用いて正常ラットに吸入インスリンを投与し，その血糖降下作用の有効性を確認することである．
【実験方法】バブルジェット式霧化装置を用いて，Wistar ラットに吸入インスリン（15U/kg），またはインスリンを含まないコントロール溶液を気管内投与した．インスリンまたはコントロール溶液投与後，0，10，20，30，60，90，120 分の血糖値を測定した．
【実験結果】吸入インスリン群の血糖値は 63 ± 10mg/dl（20 分），43 ± 8mg/dl（60 分），35 ± 9mg/dl（120 分）であった．一方，コントロール溶液群の血糖値は 80 ± 9mg/dl（$p = 0.016$），75 ± 10mg/dl（$p < 0.001$），85 ± 27mg/dl（$p < 0.001$）であった．吸入インスリン群の血糖値は，0 分と 10 分を除き全ての測定値においてコントロール溶液群より有意に低かった（$p < 0.001$）．
【結論】我々は新規バブルジェット式霧化装置を用いた吸入インスリンの投与において，その血糖降下作用の有効性を確認した．これらの結果より，新規装置はその生物活性を失うことなくインスリンを霧化できることが証明された．