附件一

行政院國家科學委員會補助專題研究計畫 □ 成 果 報 告 ■期中進度報告

金屬奈米粒子毒性及肺部之藥物控放（第 1 年）

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成果報告類型(依經費核定清單規定繳交)：■精簡報告　□完整報告

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執行單位：國立交通大學 奈米科技所

中　華　民　國　98 年　05 月　31 日

前言

The clinical experiences has led to conclusion that the current oral and intravenous therapies, albeit effective, have significant have significant short- and long-term adverse reactions that often limit use. An alternative approach is inhalation delivery of therapeutic agents. The respiratory system provides entry for the drug nanoparticles to cure both pulmonary and systemic diseases. The modern devices which are available on the market of therapeutic aerosol delivery systems have a number of disadvantages. Thus, there remains a need for an alternative means that is low cost, convenient, and capable of producing small-sized particles.

研究目的

Nanotechnology creates new possibilities to make dramatic improvements to our lives. Over the past years, nanotechnology has moved dramatically from the lab into the marketplace. Today, there are hundreds items of nanoproducts in the commercial market. The global production rate for the engineered nanomaterials was about 2500 tonnes per year in 2007 and the production rate will increase by an order of magnitude during the next 5 - 6 years [1]. The potential of nano to contribute positively to society is hard to exaggerate, but the opposite side of the coin is the potential health and environmental problems that might be caused by nano. It is shown that by different research groups that nanoparticles being inhaled can result in a serious health problems. Inhalation of Carbon Nanotubes can bring to the respiration disorder [2]. In some cases the inhalation of nanoparticles can bring to the death [3]. Nanoparticles when inhaled can enter the bloodstream and affect the central nervous system [4]. The rapidly developing field of nanotechnology is likely to become yet another source for human exposures to engineered nanoparticles. However, for the most manufactured nanoparticles no toxicity data are available. The possible harm from nanoparticles is threatening to slow the development of nanotechnology unless sound, independent and authoritative information is developed on what the risks are, and how to avoid them [5].

文獻探討

Gold is one of the most biocompatible metals. Gold nanoparticles (GNPs) are nontoxic to cultured cells; however, when injected, they brought lethal effect to mice [18-33]. We synthesized GNPs with diameters ranging from 100 nm down to 3 nm according to the published procedure [34-35]. Synthesis of GNP was monitored by UV absorbance, and the size was examined by electron microscopy. The purified GNPs were injected intraperitoneally into BALB/C mice at a dose of 8 mg/kg per week. Mice injected with 100 nm and 50 nm GNPs behaved normally and survived throughout the experimental period. Mice injected with 37 nm, 17 nm, 12 nm, and 8 nm GNPs exhibited poisoned symptoms. The treated animals showed fatigue, loss of appetite, change of fur color, and weight loss. There was a dramatic difference in the fur color of GNP-treated mice compared to normal group, typically brownish. The skin underneath had minor rashes, bruising, and hemorrhaging. Starting from day 14, mice injected with 8-37 nm GNPs significantly showed a camel-like back and crooked spine. The majority of mice in these groups died before the end of fourth week. The median survival time, defined as the length of time when half the mice died, was approximately 21 days for mice injected with 8-37 nm GNPs. Injection of 5 nm and 3 nm GNPs, however, did not induce abnormality or lethality in mice. The seemingly safe GNPs exhibited a danger zone from 8 nm to 37 nm (Fig. 1).

Pathological examination indicated an increase of Kupffer cells in liver [37], loss of structural integrity in lung, and diffusion of white pulp in spleen for the GNP-treated mice. The presence of GNP in the diseased tissues was verified by ex vivo Coherent anti-Stoke Raman scattering (CARS) microscopy [36]. The danger zone of GNPs will serve as an important parameter when employing GNP as a vehicle for drug delivery.

On the other hand, in the treatment of disease the nanoparticle inhalation represents a valuable way by which a therapeutic agent may be delivered to the human body. The administration of drugs directly into the respiratory tract has been used in a number of therapeutic areas. The field for aerosolized drug application includes treatment of lung diseases, like asthma,

chronic obstructive pulmonary disease, cystic fibrosis and lung cancer. The aerosol delivery has expanded also into the field of systemic drug delivery [6]. One of the examples of the expanding role of aerosol therapy is the development of insulin as an aerosol to treat diabetes [7, 8] The optimal target within the lungs for delivery of drugs to the systemic circulation is the alveolar region. For rapid delivery, the alveolar drug administration has a number of advantages including the large absorptive surface area, easy permeability of the alveolar walls resulting in the fast passage from the alveolar airspace to the pulmonary capillary bed, direct connection between the pulmonary circulation and the systemic circulation. The modern devices which are available on the market of therapeutic aerosol delivery systems can be subdivided into three groups which include nebulizers, dose-metering inhaler systems and dry powder inhalers. The total lung deposition efficiency for these systems normally does not exceed 10%. All these devices are able to generate the particles as small as a few microns in diameter. However, the alveolar deposition efficiency is a strong function of the particle size. The particles of size 10 to 20 nm deposit to the alveolar region about 4 times more efficiently than those several microns in diameter [9]. .

The possible alternative to nebulizers, metered-dose and dry powder inhalers is nucleation from supersaturated vapor (evaporation - condensation route [10, 11]) which combines the possibility to generate a fine aerosol with the particle diameter 1 to 100 nm and the number concentration as high as 108 cm-3 [12]. The problem is that the evaporating substance must be thermally stable. Besides, different formulations require different heating profiles. This way of aerosol delivering is waiting for the detailed investigation.

Approximately one-third of the modern drugs are water-insoluble or poorly water-soluble. Many currently available injectable formulations of such drugs can cause side effects that originate from detergents and other agents used for their solubilization. Besides, water-solubility problems delay or completely block the development of many new drugs and other biologically useful compounds. Thus, the lung deposition route can be a good alternative for the administration of poorly soluble substances. Indomethacin, which has low water solubility of 25 mg/l, is one of the

candidates considered for the lung delivery. Indomethacin is a well-known non-steroid anti-inflammatory drug for use against a wide range of diseases such as rheumatoid arthritis, spondylosis, chondrosis. However, its side effects can cause serious disorders such as bleeding and perforation of gastrointestinal tract, depression, drowse, mental disorder, increased blood pressure, congestive heart failure etc. One may hope that the aerosol lung administration of indomethacin may be an alternative route which would diminish side effects and decrease the therapeutic dose. However, new side effects like pulmonary emphysema are possible. Therefore, it is necessary to estimate the therapeutic effect as well as lung damage of indomethacin aerosol administration.

研究方法

A harm effect from Zn and Bi nanoparticles inhaled by male mice will be studied.

1) The particle flow evaporation-nucleation generators will be built for Zn and Bi nanoparticles. The dependency of particle mean diameter and number concentration on operational conditions (inert gas flow rate, evaporation temperature) will be studied to find the optimal regimes for toxicology experiments with mice.

2) The inhalation mice cameras will be constructed (basing on the previous experimental experience).

3) The particle lung deposition efficiency will be determined as a function of mean particle diameter.

4) The mice tidal volume and respiration frequency will be determined as a function of the inhalation doze.

5) The lungs histology analysis will be made for different inhalation doses.

6) Correlation between oxidative stress and different sizes of gold nanoparticles using lung cells

as model system will be studied by observing reactive oxygen species using TBARS.

7) The size-dependent oxidation will be studied by real-time RT-PCR using primer pairs against oxidative enzymes, such as SOD, HO, and interleukins.

8) ICP-MS to characterize and quantify gold nanoparticles in the cells and organs.

9) Macrophage as model system for endocytosis of gold nanoparticles.

結果與討論

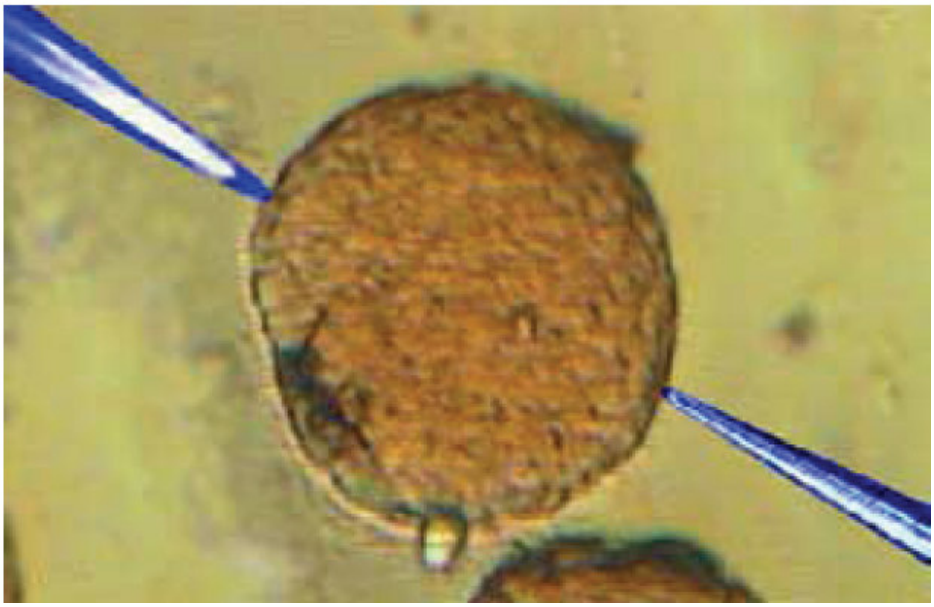

Fig. (1). An isolated neuron of Lymnaea stagnalis with microtools: microelectrode for recording membrane potential and micropipette for local application of ligand solutions. There are microelectrode inserted into the neuron (to the left of the cell) and the closely positioned micropipette (to the right of the cell). Scale bar – 5 mm.

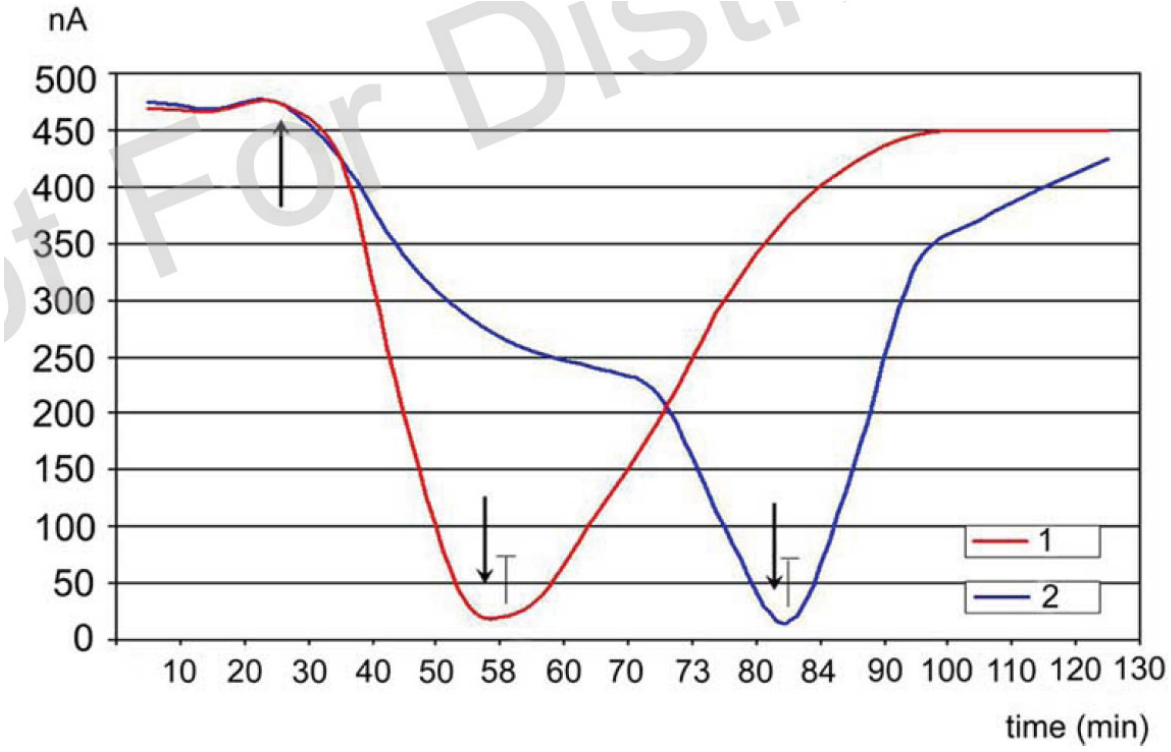

Fig. (2). Averaged changes in calcium current amplitude induced by action and washout of blockers within a group of neurons (12 cells in each group).

1. changes if amplitude of calcium currents induced by Nifedipine.

2. changes if amplitude of calcium currents induced by Nifedipine clathrate with glycyrrhizic acid.Upward arrows show start point of the blocker action. Downward arrows show start point of blockers washout. X axis set as time in minutes. Y axis set as an amplitude of incoming current in pA.

Table 1. Solubility of the NF:GA 1:4.

Saturated water solution	**Solubility of nifedipine in water**
Nifedipine	**0.18** g/l
Complex (After 10 min processing in AGO-2 planetary grinder)	**1.53** g/l (solubility of nifedipine in water increases 8.5 times)

Comparing the action of Nifedipine and the action of its clathrate with glycyrrhizic acid on isolated neurons of Lymnaea stagnalis mollusc we can conclude that clathrate reveals more affinity to receptors than basic pharmacon. This conclusion agrees with previously ascertained

property of pharmacon complexed in clathrate to manifest basic pharmacological activity at smaller dose. Hence, the origin of effect of glycoside clathration is determined by prolongation of interaction between receptor and clathrate.

Table 2. The effect of intravenous administration of NF:GK 1:4 on arterial blood pressure in Wistar rats.

Agent	Dose (mg/ kg)	Effect (decrease of arterial blood pressure in % of initial blood pressure)	Initial systolic blood pressure (mmHg)	Changes in systolic blood pressure in response to agent administration (mmHg)
Nifedipine	3.5	30	121.0± 6.6	85.0±4.3 ($p<0.05$)
Complex (0.35 mg of nifidepine)	3.5	26	141.0± 5.0	104.0± 4.3 ($p<0.05$)
Nifedipine	0.35	9	142.3± 4.4	115.0± 2.2 ($p<0.05$)
Glycyrrhizic acid	3.2	no effect	126.5± 1.3	129.6± 2.0

Table 3. The effect of intravenous administration of NF:GA 1:4 on arterial blood pressure in ISIAH rats.

Agent	Dose (mg/ kg)	Effect (decrease of arterial blood pressure in % of initial blood pressure)	Initial systolic blood pressure (mmHg)	Changes of systolic blood pressure in response to agent administration (mmHg)
Nifedipine	3.5	34	192.0± 4.0	127.0±6.0 ($p<0.05$)
Complex (0.35 mg of nifedipine)	3.5	28	180.5± 4.7	129.7±5.1 ($p<0.05$)

Complexation of glycyrrhizic acid with poorly water-soluble drugs has great potential for pharmaceutical technology and pharmaceutical dosage form design. In the NF:GA (1:4) complex solubility of nifedipine in water solutions increased to 8.5 times, effective dosage of nifedipine decreased to 10 times and pleiotropic antiarrhythmic effect obtained in very low dose of nifedipine. These effects may be explained by the GA protection of drug molecule from metabolism and prolonged drug-receptor interactions

計畫成果

期刊論文

1. Assessment of the in vivo toxicity of gold nanoparticles. Yu-Shiun Chen, Yao-Ching Hung, and Ian Liau,and G. Steve Huang, * ***Nanoscale research letters*** (2009)
2. Detection of gold nanoparticles using an immunoglobulin-coated piezoelectric sensor. Yu-Shiun Chen, Yao-Ching Hung, Kaochao Chen and Guewha Steven Huang*. ***Nanotechnology*** (2008)

會議論文

1. Yu-Shiun Chen, Yao-Ching Hung, Ian Liau, Li-Wei Lin, Meng-Yeng Hong, and G. Steve Huang*, Gold nanoparticles caused learning impairment in mice. NANO SCIENCE AND TECHNOLOGY INSTITUTE, U. S. A. (2009)
2. Yu-Shiun Chen, Ning Hung, Huei-Liang Wang, Li-Jen Tsou , and G. Steven Huang1,Nanowire Field Effect Transistor as Ultra-sensitive Biosensor,International Conference on Neuroprosthetic Device,Hsinchu , Taiwan, Republic of China (2009)
3. Chia-Hui Li, Tsung-Han Lee, Chon-Han Lee, Hsu-An Pan, Huang-Meng Chen, Yao-Ching Hong, Application of Gold Nanoparticles to Improve Site-Specific Drug Delivery in Caenorhabditis elegans Nervous System ,International Conference on Neuroprosthetic Device,Hsinchu , Taiwan, Republic of China (2009)
4. Hsu-An Pan, Chia-Wei Su, Shih-Ming Tai, Yao-Ching Hung, G.S. Huang,Surface Nanotopography Influences Implant Biocompatibility by Nanodot Arrays,International Conference on Neuroprosthetic Device,Hsinchu , Taiwan, Republic of China (2009)
5. Y.-P. Sui, S.-A. Pan, S.-M.Tai,Y.-C. Hung, G.-S. Huang,Nanostructured electrode surfaces used for implantable neural device to improve biocompatibility for muscle cell,International Conference on Neuroprosthetic Device,Hsinchu , Taiwan, Republic of China (2009)
6. Wei-shu Lin, Yu-Shiun Chen, Y.-C. Hung and G. Steven Huang,The Immunogenic Property of Gold Nanoparticles,International Conference on Neuroprosthetic Device,Hsinchu , Taiwan, Republic of China
7. Shih-Ming Tai, Hsu-An Pan, Yu-Ping Sui,Yao-Ching Hung, G.Steven Huang,Customized Nanodot Arrays Provide an Appropriate Platform for Neural Medical Device in Spinal Cord,International Conference on Neuroprosthetic Device,Hsinchu , Taiwan, Republic of China (2009)
8. Yao-Ching Hung, Huang-Meng Chenb, Li-Ko Yeh, Meng-Yen Hong, and Guewh.Steven Huang,Electromagnetic fields induce apoptosis and affect neuron,International Conference on Neuroprosthetic

References

1. Nanotechnology: A research strategy for addressing risk. A. D. Maynard, Project on Emerging Nanotechnologies supported by THE PEW CHARITABLE TRUSTS.
2. Warheit, D.B. et al., 2004."Comparative Pulmonary Toxicity Assessment of Single-Wall Carbon Nanotubes in Rats," Toxicological Sciences, 77:117–125
3. Oberdörster G, Gelein RM, Ferin J, Weiss B. Association of particulate air pollution and acute mortality: involvement of ultrafine particles? Inhal Toxicol. 1995 7(1):111–124.
4. Elder A, Gelein R, Silva V, Feikert T, Opanashuk L, Carter J, Potter R, Maynard A, Ito Y, Finkelstein J, Oberdörster G. 2006. Translocation of Inhaled Ultrafine Manganese Oxide Particles to the Central Nervous System. Environ Health Perspect. Apr; 114 (8): 1172-1178.
5. A. D. Maynard R. J. Aitken, T. Butz, V. Colvin, K. Donaldson, G. Oberdцrster, M. A. Philbert, J. Ryan, A. Seaton, V. Stone, S. S. Tinkle, L. Tran, N. J. Walker and D. B. Warheit, Safe handling of nanotechnology, Nature, 2006, 444 (16), 267 - 269
6. Laube, B. L. 2005. The expanding role of aerosols in systemic drug delivery, gene therapy, and vaccination. *Respiratory Care* 50: 1161 - 1176.
7. Skyler, J. S. 2007. Pulmonary insulin delivery—state of the art 2007. *Diabetes Technology & Therapeutics* 9: S1 - S3.
8. BRAIN, J. D. 2007. Inhalation, deposition, and fate of insulin and other therapeutic proteins. *Diabetes Technology & Therapeutics* 9:, S4 - S15.
9. Oberdörster, G., E. Oberdörster, and J. Oberdörster. 2005. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. *Environmental Health Perspectives* 113: 823 - 839.
10. Rabinowitz, J. D., M. Wensley, P. Lloyd, D. Myers, W. Shen, A. Lu, C. Hodges, R. Hale, D. Mufson, and A. Zaffaroni. 2004. Fast onset medications through thermally generated aerosols. *The Journal Of Pharmacology And Experimental Therapeutics* 309: 769–775,
11. Byron, P. R. 2004. Drug delivery devices. issues in drug development. *Proc. Am. Thorac. Soc.* 1: 321–328.
12. Fuchs N. M. 1964. The Mechanics of Aerosol. Oxford, Pergamon press.

We are planning going to your institute for a visit in this year. Professor Tsai suggested that May is a good month to go. So, we were targeting May for the first approximation.

Although there was only few communications between us, we preceded experiments regarding to *the toxicity of gold nanoparticles* and *nano-surface using cultured lung cells as working mode*l. There have been some interesting results. Maybe we could exchange our findings when we meet.

Our lab is focusing on the biocompatibility of nanostructures, including nanoparticles and nano-surface. The overall goal is to understand the biocompatability of nanostructure at the molecular and cellular level. And, if possible, apply these rules and make use of nanostructures in medicine, especially in artificial implants and drug delivery.

Background. Aerosol lung administration is a convenient way to deliver water-insoluble or poorly soluble drugs, provided that small-sized particles are generated. Here for the outbred male mice we show that the pulmonary administration of ibuprofen nanoparticles requires a dose which is 3 to 5 orders of magnitude less than that for the orally delivered particles at the same analgesic effect.

Methods. The aerosol evaporation - condensation generator consisted of a horizontal cylindrical quartz tube with an outer heater. Argon flow was supplied to the inlet and aerosol was formed at the outlet. The particle mean diameter and number concentration varied from 10 to 100 nm and 103 – 107 cm^{-3}, respectively. The analgesic action and side pulmonary effects caused by the inhalation of ibuprofen nanoparticles were investigated. The chemical composition of aerosol

particles was shown to be identical with the maternal drug. Using the nose-only exposure chambers, the mice lung deposition efficiency was evaluated as a function of the particle diameter. The dose-dependent analgesic effect of aerosolized ibuprofen was studied in comparison with the oral treatment.

Results and conclusions. It was found that the dose for aerosol treatment is three to five orders of magnitude less than that required for oral treatment at the same analgesic effect. Accompanying effects were moderate venous hyperemia and some emphysematous signs.

The analgesic action and side pulmonary effects caused by the inhalation of ibuprofen nanoparticles 10 - 100 nm in diameter were investigated. The nanoparticles were formed by the evaporation - condensation route. The chromatographic and UV analysis showed that the aerosol particles were chemically identical to the maternal substance (i.e. there was no thermal decomposition or oxidation during evaporation). The X-ray diffraction analysis showed that the nanoparticle crystal phase (racemic ibuprofen) was identical to that of the original ibuprofen powder.

Using the nose-only exposure chambers, the mice lung deposition efficiency was evaluated as a function of the particle diameter changing from about unity at d = 10 nm to about 0.2 at d = 100 nm.

The dose-dependent effect of aerosolized ibuprofen was studied in comparison with the oral treatment. It was found that aerosol administration is much more effective than the oral delivery; thus, the aerosol treatment needs the dose a three to five orders of magnitude less than the oral one at the same analgesic effect. From 25 / 04 / 2009 To 02 / 05 / 2009