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Introduction of poly-L-lactic acid microspheres into the skin using supersonic flow: effects of helium gas pressure, particle size and microparticle dose on the amount introduced into hairless rat skin

Masaki Uchida, Yi Jin, Hideshi Natsume, Daisuke Kobayashi, Kenji Sugibayashi and Yasunori Morimoto

Abstract

A microparticulate bombardment system loaded with DNA- and RNA-coated gold and tungsten microparticles (diameter 1–3 μm; density about 19 g cm^{-3}), the Helios gene gun system (Helios gun system), has been used to deliver a gene into cells by accelerating the microparticles to high velocity using a supersonic flow of helium gas. To investigate whether drug-loaded microspheres, >20 μm in diameter and about 1.0 g cm^{-3} in density, could be delivered in powder form quantitatively into the skin using the Helios gun system equipped with a cartridge container fitted with a rupture membrane, we investigated the effect of the helium gas pressure in accelerating indometacin-loaded poly-L-lactic acid (PLA) microspheres, as well as the particle size and the bombardment dose on delivery into the skin. Introduction of indometacin (i.e. indometacin-loaded PLA microspheres) after bombardment, with 3.0 mg indometacin-loaded PLA microspheres of a particle size of 20–38, 44–53 and 75–100 μm at a helium pressure of 100, 200 and 300 psi, of the abdomen of hairless rats increased in parallel with the helium pressure and it was also affected by the particle size, being highest at a diameter of 75–100 μm. However, introduction of higher amounts of PLA microspheres resulted in more severe skin erythema (skin damage) as monitored by the Draize score. Using lower bombardment doses (0.5 and 1.0 mg), the efficiency of introduction was improved and the skin damage markedly reduced. Moreover, discrete bombardment with a low dose provided a more efficient introduction of indometacin and less skin damage. These results suggest that bombardment injection of drug-loaded microspheres in a powdered form by the Helios gun system appears to be a very useful tool for the quantitative delivery of a variety of drugs and an alternative to parenteral injection by needle, especially for delivering water-soluble macromolecules.

Faculty of Pharmaceutical Sciences, Josai University, Sakado, Saitama 350-0295, Japan

Masaki Uchida, Hideshi Natsume, Daisuke Kobayashi, Kenji Sugibayashi, Yasunori Morimoto

College of Pharmacy, Zhejiang University, Hangzhou 310031, China

Yi Jin

Research Institute of TTS Technology, Josai University, Sakado, Saitama 350-0295, Japan

Hideshi Natsume, Kenji Sugibayashi, Yasunori Morimoto

Correspondence: Y. Morimoto, Faculty of Pharmaceutical Sciences, Josai University, 1-1 Keyakidai, Sakado, Saitama 350-0295, Japan. E-mail: morimoto@josai.ac.jp

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Introduction

The potential for using the skin as an alternative route for administering systemically active drugs has attracted considerable interest in recent years. However, the stratum corneum, which is responsible for the impermeable nature of the skin, is well known to act as a protective barrier against the loss of physiologically essential substances and to prevent the diffusion of potentially toxic chemicals from the external environment into the body. The stratum corneum has also been shown to be the major rate-limiting barrier to drug diffusion and penetration through and into the skin. Hence, many drugs have ceased to be

developed for transdermal delivery due to this barrier in the absence of chemical penetration enhancers (Sugibayashi et al 1985; Manabe et al 1996) or other physical means (i.e. iontophoresis (Tyle 1986; Green et al 1991), or phonophoresis (Ueda et al 1995; Asano et al 1997)) being incorporated into the delivery system. In particular, water-soluble peptide and protein drugs definitely require some sort of penetration enhancement technology because the size and charge of the molecules themselves hinder absorption.

Recently, a microparticulate bombardment system, the Helios gene gun system, has been introduced to deliver genes into mammalian and plant cells (Novakovic et al 1999; Oshikawa et al 1999). This system is able to introduce DNA- and RNA-coated gold and tungsten microparticles into living cells following acceleration to a high velocity using a supersonic flow of helium gas. Also, this system can deliver these microparticles directly in powder form. Consequently, the gene expression (Thompson et al 1993; Heiser 1994) was much higher than that obtained by other systems using diethylaminoethyl (DEAE)-dextran (Luthman & Magnusson 1983), lipofectin (Felgner et al 1987; Ponder et al 1991) and electroporation (Chu et al 1987; Weaver 1995). In addition, the successful introduction of DNA and RNA via the skin has been achieved in-vivo in mice (Chang et al 1998), rats (Cheng et al 1993) and pigs (Macklin et al 1998). Therefore the microparticulate bombardment system is also able to deliver a variety of drugs into the skin because it circumvents the rate-limiting barrier of the stratum corneum.

Microparticles consisting of gold or tungsten, 1–3 μm in diameter, were used with a density of about 19 g cm^{-3} (Sanford et al 1987; Heiser 1994). These can be used to introduce DNA and RNA into cells using the Helios gene gun system because the velocity of particles bombarded from this system is determined by the particle density, thereby resulting in the highly efficient introduction of these microparticles. However, these particles were found to be unsuitable for delivering a variety of drugs into the skin because of their non-biodegradable nature and subsequent accumulation in the body. In addition, the coating method used to bind DNA and RNA to the surface of microparticles was unable to support a constant loading of drugs and control the drug release and dissolution. Larger biodegradable microparticles can be used to overcome these problems because larger microparticles, prepared using biodegradable materials, are heavier than smaller microparticles, and so take the place of the high-density gold and tungsten used to prepare smaller microparticles.

In this study, we first carried out a histological examination to see if larger microparticles with a density of 1.0 g cm^{-3} could be introduced into the skin using the Helios gene gun system (Helios gun system). Fluorescein isothiocyanate (FITC)-labelled polystyrene microspheres with a mean diameter of 45 μm were used to evaluate the introduction of larger microparticles into the skin using confocal laser scanning microscopy. Biodegradable microspheres with different diameters (20–38, 44–53 and 75–100 μm) were prepared using poly-L-lactic acid (PLA). Indometacin was used as a water-insoluble model drug to estimate the percentage introduction of indometacin-loaded PLA microspheres into the skin after bombardment with PLA microspheres using the Helios gun system. The effect of helium gas pressure to accelerate the microspheres, and the particle size, on the percentage introduction into the skin and the degree of skin damage in terms of a Draize score (Draize et al 1944) were investigated after PLA microsphere bombardment. Based on these results, the effects of the bombardment dose of PLA microspheres and discrete bombardment were also evaluated.

Materials and Methods

Materials

Indometacin and rhodamine 6G were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Poly-L-lactic acid (PLA; MW approx. 10000) was obtained from Nacalai Tesque, Inc. (Kyoto, Japan). Fluorescein isothiocyanate (FITC)-labelled polystyrene microspheres (mean diameter 45 μm) were obtained from Polysciences, Inc. (Warrington, PA). A polyvinyl chloride film (thickness 10 μm) was purchased from Hitachi Chemical Filtec Inc. (Tokyo, Japan). All other chemicals were of reagent grade.

Animals

Male WBN/ILA-Ht hairless rats (body weight 240–290 g) were supplied by the Life Science Research Center of Josai University (Saitama, Japan). All experimental protocols met the Guidelines for Animal Experimentation approved by the Japanese Association of Laboratory Animal Science and the Japanese Pharmacological Society. The rats were maintained at 24$\pm$1°C under a 12-h light–dark cycle and had free access to a standard rodent diet and clean water. All experiments was carried out under anaesthesia (25% w/v urethane, 1 mg kg^{-1}, i.p.). The rats were sacrificed by slowly increasing the CO_2 concentration in the CO_2 gas animal

euthanasia cabinet (KN-750-1, Natsume Co. Ltd, Tokyo, Japan) after finishing the experiments.

Introduction of drug-loaded microspheres into the skin using the Helios gun system

A novel cartridge container, consisting of a stainless-steel ring (length 1.5 cm, diameter 3 mm), was assembled with a rupture membrane and a stainless-steel ring (i.d. 2 mm, o.d. 3 mm) was used to keep precise amounts of drug-loaded microspheres in the cartridge container. A polyvinyl chloride film (thickness 10 μm) was used as a rupture membrane. Briefly, the rupture membrane was sandwiched between the stainless-steel tube and ring, then precise amounts of drug-loaded microspheres were transferred to the cartridge container. The cartridge containers filled with drug-loaded microspheres were set on a cartridge holder which had twelve holes. The cartridge holder was then inserted into the Helios gun system. The bombardment from microspheres in the cartridge container was initiated by pressing the trigger button which opens the main helium gas valve. The polyvinyl chloride film at the bottom of the cartridge container was ruptured easily following bombardment with microspheres. Consequently, very few microspheres remained in the cartridge container (less than 1%); the novel cartridge container with the rupture membrane functioned well as far as the bombardment with microspheres was concerned.

Confocal laser scanning microscopic observations after bombardment of skin with FITC-labelled polystyrene microspheres

After anaesthetizing (urethane 1 mg kg^{-1} i.p.) male hairless rats, 3.0 mg FITC-labelled polystyrene microspheres (mean diameter 45 μm) in the cartridge container were bombarded using the Helios gun system. The skin area used was less than 25 mm in diameter because the diameter of the barrel liner connected to the tip of the Helios gun system was 25 mm (about 5 cm^2). It was thought that bombarded microspheres would be likely to concentrate in the centre of the application site (as judged from the skin erythema, see Figure 3). The helium pressures used were 100, 200 and 300 psi. The skin was then excised and frozen at -20°C in a Microtome Cryostat (Damon/Ice Divison, MA), while fixed on a platform using medical gel (Tissue-Teck, Sakura Finetechnical Co. Ltd, Tokyo, Japan). The skin was cut into slices about 30 μm thick using a microtome to obtain cross-sections and each of these was transferred to a glass slide. Then, rhodamine 6G (0.5 mM) was added to the cross-section. After 5 min, fluorescence from FITC-labelled polystyrene microspheres and rhodamine 6G was monitored by confocal laser scanning microscopy (CLSM, MRC-600 Lasersharp System, Bio-Rad Laboratories, Richmond, CA). CLSM linked to a Zeiss Axioplan equipped with a Zeiss Neofluar (Carl Zeiss, Oberkochen, Germany) was then used for confocal imaging. The fluorescence of the FITC-labelled polystyrene microspheres and rhodamine 6G was measured at an excitation wavelength of 488 and 514 nm and an emission wavelength of 515 and 550 nm, respectively. Data were processed with a desk-top computer linked to an optical disk drive using Comos software (Bio-Rad Laboratories). All experiments were carried out in triplicate.

Preparation of indometacin-loaded PLA microspheres

Indometacin-loaded PLA microspheres were prepared by an o/w solvent evaporation method (Gohel et al 1996). Briefly, 100 mg indometacin and 300 mg PLA were dissolved in 3 mL dichloromethane (CH_2Cl_2). This solution was then added to 150 mL cold 0.5% polyvinyl alcohol aqueous solution while stirring at 2500 rev min^{-1}. The mixture was then stirred at 2000 rev min^{-1} for 25 min at room temperature. Thereafter, the mixture was continuously stirred at 1000 rev min^{-1} for 5 h at 37°C to evaporate CH_2Cl_2. The hardened indometacin-loaded PLA microspheres were collected by centrifugation (3500 rev min^{-1}) and washed 6 times with distilled water. After freeze-drying, the harvested PLA microspheres were divided into the required particle size ranges using stainless-steel mesh sieves (diameter 20–38, 44–53 and 75–100 μm). The size distribution in all three kinds of sieved PLA microspheres was measured. The mean particle size in PLA microspheres of 20–38, 44–53 and 75–100 μm in diameter were 30.3 ± 7.33, 49.8 ± 6.41 and 92.2 ± 12.6 μm, respectively (volume-weighted mean $\pm$ s.d.).

The density of the indometacin-loaded PLA microspheres with different size ranges was measured by liquid displacement (Martin et al 1993). The density of microspheres was about 1.2 g cm^{-3} for all size ranges.

Determination of indometacin content in PLA microspheres

Five milligrams of indometacin-loaded PLA microspheres was weighed precisely and dissolved in 5 mL acetone and the solution diluted with acetonitrile to a

total volume of 50 mL. Then, 200 μL of this mixture was added to 200 μL acetonitrile containing n-hexyl *p*-hydroxybenzoate (Tokyo Chemical Industry Co., Tokyo, Japan) as an internal standard. The amount of indometacin in the solution was analysed by HPLC to determine the indometacin content of the PLA microspheres. The HPLC system used involved a pump (LC-10S), a UV detector (SPD 10A), an integrator (C-R5A), a column oven (CTO-10A), a system controller (SCL-10A), an auto injector (Sil-10A) (Shimadzu, Kyoto, Japan), and a reverse-phase column (Inertsil ODS-2, 5 μm, 4.6 × 250 mm; GL Sciences Inc., Tokyo, Japan). The mobile phase was 0.1% phosphoric acid–acetonitrile (45:55, v/v) and the flow rate was 1.2 mL min^{-1}. The UV detector was operated at 262 nm and the column temperature was maintained at 40°C. The indometacin content of PLA microspheres with a diameter of 20–38, 44–53 and 75–100 μm was 10.4, 18.8 and 22.2%, respectively.

Effect of helium pressure and particle size on introduction

After anaesthetizing (urethane 1 mg kg^{-1} i.p.) male hairless rats, 3.0 mg indometacin-loaded PLA microspheres (diameter 20–38, 44–53 and 75–100 μm) was transferred to the cartridge container and accelerated by high velocity helium gas in the Helios gun system and then applied to the shaved abdominal skin. The skin area involved was less than 25 mm in diameter. The helium pressures used were 100, 200 and 300 psi, respectively.

Effect of bombarded dose on introduction

After anaesthetizing (urethane 1 mg kg^{-1} i.p.) male hairless rats, 0.5 and 1.0 mg indometacin-loaded PLA microspheres (diameter 75–100 μm) were bombarded on to the skin at a helium pressure of 100, 200 and 300 psi as described above.

Determination of amount of indometacin introduced into the skin

After bombardment with indometacin-loaded PLA microspheres as described, the skin (diameter 2.5 cm, area approx. 5 cm^2) was excised immediately, and the surface washed and cleaned with physiological saline (pH 7.2). The excised skin was homogenized with an adequate amount of pH 7.4 phosphate-buffered saline (PBS; 2.5 and 5.0 mL) in a homogenizer (Polytron PT 3000, Kinematica AG, Switzerland). The resulting skin homogenate was mixed with an adequate amount of ice-cold acetone–acetonitrile (1:4) to give a volume of 7.5 mL and 15.0 mL and then shaken for 5 min. The supernatants were collected following centrifugation at 18000 *g* at 4°C for 5 min and then stored in a refrigerator until use.

Supernatants containing indometacin (200 μL) were mixed with 200 μL of acetonitrile containing n-hexyl *p*-hydroxybenzoate as an internal standard. The amount of indometacin in the mixture (50 μL) was determined by HPLC as described above.

The percentage introduction of indometacin into the skin was calculated as follows:

$$\% \text{ Introduction} = (\text{Amount of indometacin entering skin}/\text{Loading dose of indometacin}) \times 100 \quad (1)$$

where the loading dose of indometacin is the indometacin content of the PLA microspheres in the cartridge container. The recovery of indometacin from skin lysate was almost complete (99.8 ± 0.66%, n = 6).

Effect of discrete bombardment on introduction

After anaesthetizing (urethane 1 mg kg^{-1} i.p.) male hairless rats, 1.0 mg indometacin-loaded PLA microspheres (diameter75–100 μm) was transferred to each cartridge container and accelerated by a high velocity stream of helium gas in the Helios gun system, and then applied to the shaved abdominal skin at three different sites. The helium pressure used was 300 psi. The skin (3 sections, diameter 2.5 cm, area about 5 cm^2) was excised immediately after bombardment with PLA microspheres. It was then treated as described above. The skin was homogenized with 15.0 mL pH 7.4 PBS and then mixed with acetone–acetonitrile (1:4) to give a final volume of 45.0 mL.

Evaluation of skin erythema

Indometacin-loaded PLA microspheres, 20–38, 44–53 and 75–100 μm in diameter, were used to bombard the abdomen of anaesthetized (urethane 1 mg kg^{-1} i.p.) male hairless rats using the Helios gun system at doses of 0.5, 1.0 and 3.0 mg, and helium pressures of 100, 200 and 300 psi, respectively. After 1 h of bombardment, the skin erythema was evaluated using the Draize score

(Draize et al 1944). The scores were as follows: 0, no erythema; 1, very slight (barely perceptible); 2, well defined; 3, moderate to severe; 4, severe. The score represents the range (n = 3–15).

Photographs of the abdomen were taken by a digital camera (Fine Pix 700, Fujifilm, Japan) 1 h after bombardment with indometacin-loaded PLA microspheres (diameter 75–100 μm), at doses of 0.5, 1.0 and 3.0 mg, and discrete bombardment of three different sites at a dose of 1.0 mg.

Statistics

Statistical analyses were performed using one-way analysis of variance followed by Fisher's pairing *t*-test to assess significant differences between the treatment groups. Statistical analyses to examine the effects of helium pressure, particle size and bombardment dose on the Draize score were performed using Friedman test. A *P*-value of 0.05 was considered significant.

Results and Discussion

CLSM observation of the cross-section of hairless rat skin after bombardment with FITC-labelled polystyrene microspheres

Figure 1 shows a typical confocal laser scanning microphotograph of the cross-section of hairless rat skin immediately after bombardment with FITC-labelled polystyrene microspheres (mean diameter 45 μm) using the Helios gun system (helium pressure 300 psi). It can be seen that microspheres were introduced into the skin. Microspheres were also introduced at helium pressures of 100 and 200 psi (data not shown), suggesting that the Helios gun system with its novel cartridge container is able to introduce larger microspheres into the skin in a similar fashion to smaller gold particles (diameter 1–3 μm) (Andree et al 1994). Microspheres were observed in deeper areas at higher helium pressure, most microspheres being found in the epidermis (including the stratum corneum) at a helium pressure of 100 psi, whereas microspheres were found in both the dermis and epidermis at 200 and 300 psi. Also, the number of microspheres in the skin tended to increase with increasing helium gas pressure. These results indicate that the Helios gun system can deliver larger amounts of microspheres in powder form into deeper regions of the skin if a higher helium pressure is used.

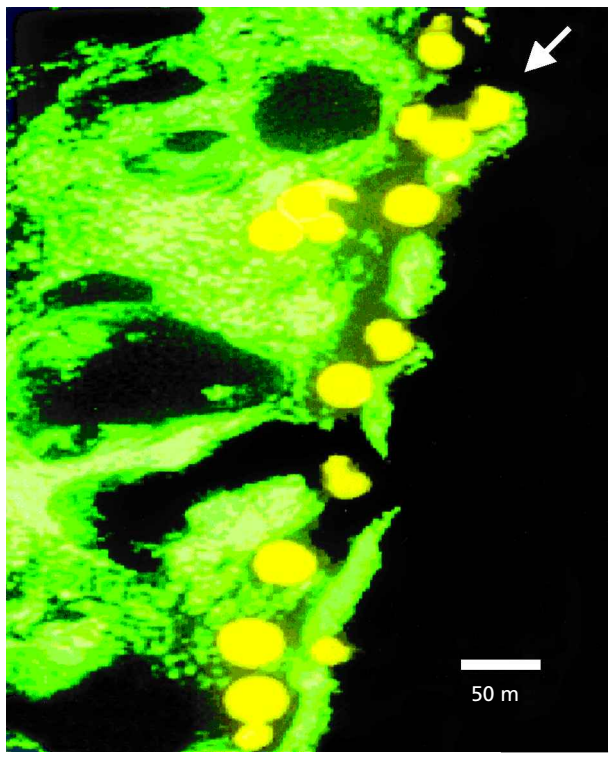

Figure 1 Confocal laser micrographs of hairless rat skin after bombardment with FITC-labelled polystyrene microspheres (mean diameter, 45 μm) using the Helios gun system (helium pressure 300 psi). Arrow indicates FITC-labelled polystyrene microspheres.

Effects of helium gas pressure and particle size on the percentage introduction of indometacin and skin erythema after bombardment using the Helios gun system

Figure 2 shows the effect of helium pressure and particle size of indometacin-loaded PLA microspheres on the percentage introduction of indometacin into the skin after bombardment with indometacin-loaded PLA microspheres at a dose of 3.0 mg in hairless rats. The percentage introduction is listed in Table 1. When indometacin-loaded PLA microspheres with different size ranges were bombarded at various helium pressures, indometacin was introduced into the skin in all cases, thereby allowing introduction of PLA microspheres. At a helium pressure of 100 and 200 psi, there was no significant difference between the percentage introduction of indometacin after bombardment with PLA microspheres of different size ranges ($P > 0.05$, Fisher's pairing *t*-test). One possible reason is that the particle size effect (mass effect) may be insufficient at these pressures. Further investigation will be required to clarify this. In contrast, the percentage introduction at

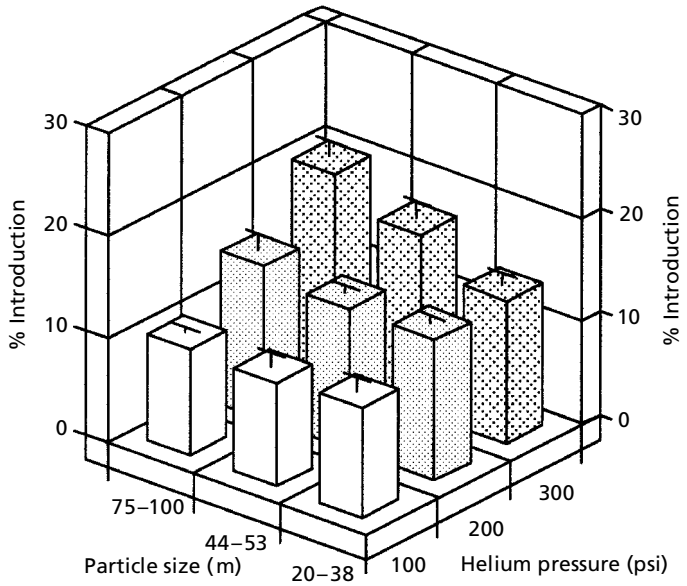

Figure 2 Relationship between helium gas pressure, particle size and the percentage introduction of indometacin into the skin of hairless rats after bombardment with indometacin-loaded PLA microspheres using the Helios gun system. Bombardment dose was 3.0 mg. There was a significant difference between the percentage introduction obtained at different helium pressures in particle size of 44–53 and 75–100 μm ($P < 0.05$, one-way analysis of variance). There was no significant difference between the percentage introduction obtained with different particle sizes at all helium pressures ($P > 0.05$, one-way analysis of variance). There was a significant difference between the percentage introduction after bombardment with PLA microspheres of 20–38 and 75–100 μm in diameter at a helium pressure of 300 psi ($P < 0.05$, Fisher's pairing t-test). Each column represents the mean and standard error (n = 3 or 4).

300 psi was increased with increasing particle size; there was no significant difference between the percentage introduction obtained with different particle sizes of PLA microspheres ($P > 0.05$, one-way analysis of variance), but a significant difference between the percentage introduction after bombardment with PLA microspheres of 20–38 and 75–100 μm was observed ($P < 0.05$, Fisher's pairing t-test). Since smaller-size microparticles (diameter 20–38 μm) were found to have a relatively large range (see Materials and Methods), larger microparticles in this size range were found to be introduced more easily into the skin, thereby allowing no difference between the percentage introduction after bombardment with microparticles of 20–38 and 44–53 μm in size. In addition, since higher pressure was found to provide a more effective velocity of microspheres in all particle size ranges, the percentage introduction might be increased with increasing particle size (i.e. particle mass). Moreover, the percentage introduction tended to increase depending on helium pressure at all particle size ranges (for 44–53 and 75–100 μm diameter: $P < 0.05$, one-way analysis of variance). These results indicate that the percentage introduction of indometacin into the skin after bombardment with indometacin-loaded PLA microspheres is highly dependent on the helium pressure, associated with the microspheres' velocity following acceleration by helium gas, and is also affected by the particle size at a helium pressure of 300 psi. In addition, a controlled release may be possible for the delivery of drug via microspheres into the systemic circulation, if a suitable biodegradable material for the walls of the microspheres is selected.

Table 1 shows the Draize score 1 h after bombardment of skin of hairless rats with indometacin-loaded PLA microspheres at a dose of 3.0 mg at different size ranges and different helium pressures. Skin erythema was observed at all helium pressures used. This erythema was found to originate in the capillary burst under stereoscopic examination. The Draize score tended to increase on increasing the helium pressure at all particle size ranges, although there was no significant difference between the Draize scores obtained at different helium pressures and with different particle sizes of PLA microspheres ($P > 0.05$, Friedman test). On the other hand, the score obtained with different size ranges of microspheres was almost identical at the same helium pressure. It seems, therefore, that greater introduction of indo-

Table 1 Percentage introduction and Draize score range after bombardment of IDM-loaded PLA MS using the helios gun system under various conditions.

Particle size range	Helium pressure	% introduction	Draize score range
At a dose of 3.0 mg			
20–38 μM	100 psi	10.6±1.74	1–1
	200 psi	13.6±0.87	1–2
	300 psi	13.9±1.35	2–3
44–53 μM	100 psi	10.0±1.76	0–0
	200 psi	13.6±0.75	1–2
	300 psi	17.3±1.82	2–3
75–100 μM	100 psi	10.3±0.30	0–1
	200 psi	14.8±1.81	1–2
	300 psi	20.1±1.73	2–3
At a dose of 1.0 mg			
75–100 μM	100 psi	30.4±6.00	0–0
	200 psi	33.8±5.17	1–1
	300 psi	39.6±3.04	1–2
At a dose of 0.5 mg			
75–100 μM	100 psi	35.4±2.97	0–0
	200 psi	39.3±4.29	1–1
	300 psi	35.6±4.16	1–2

There was a significant difference between the % introductions obtained at different bombardment doses at all helium pressures (one-way analysis of variance, $P < 0.01$). There was no significant difference between the Draize scores obtained at different helium pressures with different particle sizes and at different bombardment doses of PLA MS (Friedman test, $P > 0.05$). Each data point represents the mean and standard error, and the range (n = 3–15).

metacin into the skin achieved at higher helium pressures tends to lead to a higher Draize score in most cases. In addition, the erythema was reduced with the passage of time at all helium pressures and had disappeared 2 days after bombardment (data not shown).

We conclude that the larger microspheres and higher helium pressures used in this study lead to a better introduction of indometacin into the skin, accompanied by greater damage to the skin.

Effect of bombardment dose of indometacin-loaded PLA microspheres on the percentage introduction of indometacin and skin erythema

To improve indometacin introduction and reduce skin damage, the effect of the bombardment dose of indometacin-loaded PLA microspheres on the percentage introduction of indometacin and skin damage was investigated. Table 1 shows the percentage introduction and the Draize score after bombardment with indometacin-loaded PLA microspheres (diameter 75–100 μm) at doses of 0.5 and 1.0 mg, and different helium pressures. There was a significant difference between the percentage introduction obtained at different bombardment doses including 3.0 mg at all helium pressures ($P < 0.01$, one-way analysis of variance). The percentage introduction of indometacin into the skin was increased in parallel with the bombardment dose at all helium pressures, although the percentage introduction was almost the same for 0.5 and 1.0 mg at 300 psi ($P > 0.05$, Fisher's pairing t-test). The percentage introduction at a dose of 0.5 and 1.0 mg was 2–3 times higher than that at a dose of 3.0 mg ($P < 0.01$, Fisher's pairing t-test). This same trend was also observed after bombardment with indometacin-loaded PLA microspheres with diameters of 20–38 and 44–53 μm (data not shown). No helium pressure-dependence for the percentage introduction was observed with bombardment doses of 0.5 and 1.0 mg. This is most likely due to reduced particle collisions and a reduction in the coefficient of friction between the particles and the wall of the cartridge container, thereby allowing a higher particle velocity, so providing a higher introduction. In addition, the larger microspheres used at lower doses tended to increase the percentage introduction of indometacin, particle size ranges of 75–100 μm generally

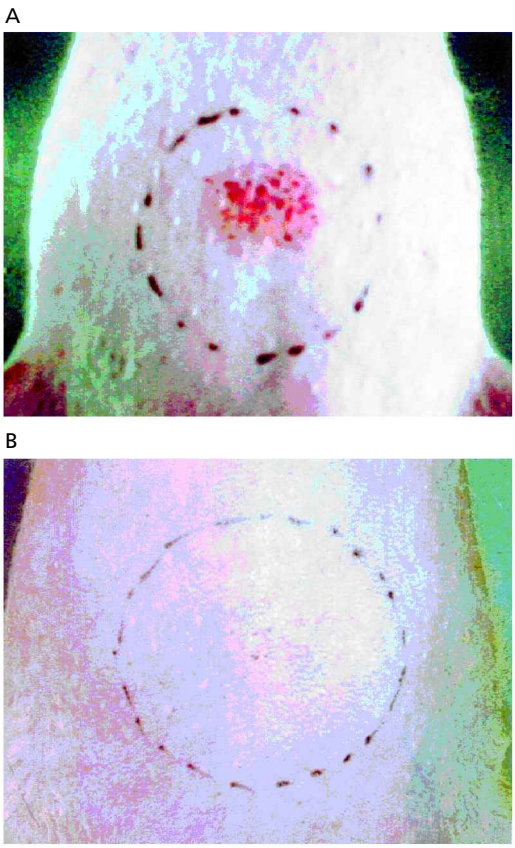

Figure 3 Photographs of the abdomen of a hairless rat 1 h after bombardment with indometacin-loaded PLA microspheres using the Helios gun system. Bombardment dose, 3.0 mg (A) or 0.5 mg (B); particle size, 75–100 μm; helium pressure, 300 psi.

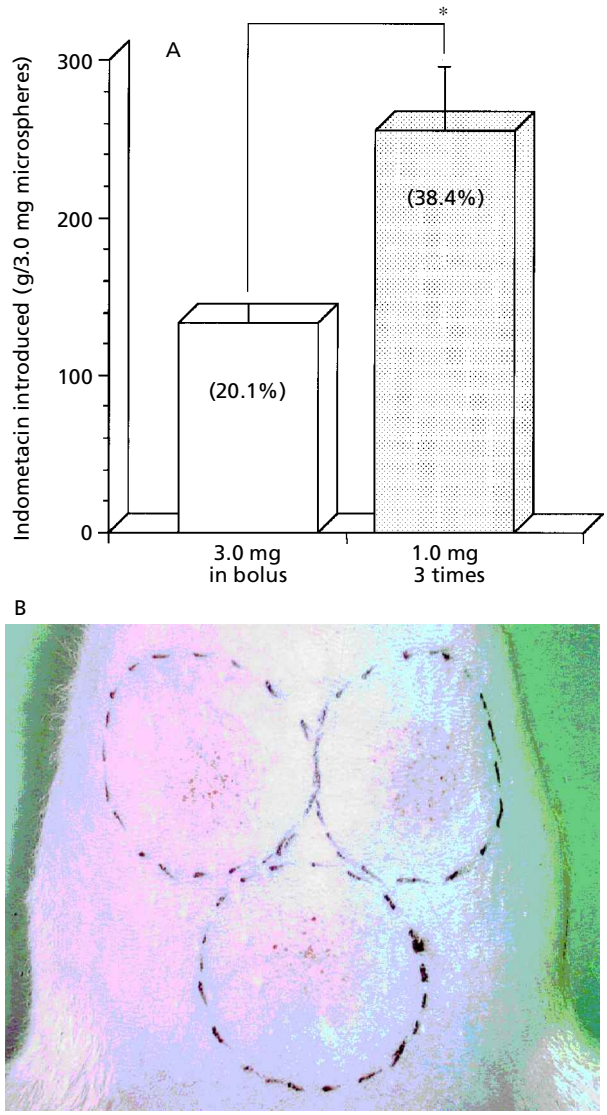

Figure 4 Amount of indometacin introduced into the skin (A) and photograph of the abdomen of a hairless rat (B) after discrete bombardment of indometacin-loaded PLA microspheres using the Helios gun system. Bombardment method, 3.0 mg microspheres in bolus or 1.0 mg microspheres ×3; particle size, 75–100 μm; helium pressure, 300 psi. Numbers in parentheses indicate the percentage introduction of indometacin into the skin. *$P < 0.05$, Fisher's pairing *t*-test, vs bolus dose. Each data column represents the mean and standard error (n = 4 or 5).

being more efficient than those of 20–38 and 44–53 μm (data not shown). These results suggest that a reduction in the bombardment dose may lead to a more efficient introduction, although the amount of indometacin introduced fell substantially on reducing the bombardment dose.

On the other hand, at all helium pressures, the Draize scores at doses of 0.5 and 1.0 mg were much lower than those at a dose of 3.0 mg at all helium pressures (Table 1), although there was no significant difference between the Draize scores obtained at different bombardment doses of PLA microspheres and helium pressures ($P > 0.05$, Friedman test). Reduction in helium pressure also led to a reduction in the Draize score for all particle size ranges. Figure 3 shows typical photographs of the abdomen of hairless rats after bombardment with indometacin-loaded PLA microspheres (diameter 75–100 μm, helium pressure 300 psi), at different doses. It is clear that the degree of skin erythema after bombardment at a dose of 0.5 mg (Figure 3B) is very minor compared with that at a dose of 3.0 mg (Figure 3A). The bombardment at a dose of 1.0 mg also resulted in a minor degree of erythema (Figure 4B). The same trend was observed after bombardment with indometacin-loaded PLA microspheres, 20–38 and 44–53 μm in di-

ameter. These results indicate that a reduction in the bombardment dose leads to less skin damage.

Overall, we concluded that bombardment with larger microspheres at a lower dose provides a more efficient introduction of indometacin and less skin damage. However, the amount of indometacin introduced at doses of 0.5 and 1.0 mg was lower than that at a dose of 3.0 mg.

Effect of discrete bombardment on the amount of IDM introduced into the skin

To increase the amount of indometacin introduced, the effect of discrete bombardment with indometacin-loaded PLA microspheres was investigated because of the higher introduction of indometacin by bombardment with smaller amounts of PLA microspheres described above. Figure 4 shows the amount of indometacin introduced into the skin and a photograph of the abdomen of a hairless rat after discrete bombardment with indometacin-loaded PLA microspheres (diameter 75–100 μm; helium pressure 300 psi). When each 1.0 mg of indometacin-loaded PLA microspheres was used to bombard three different sites of the abdomen, the total amount of indometacin introduced into the skin was significantly higher than that after bombardment with a bolus dose of 3.0 mg (Figure 4A; $P < 0.05$, Fisher's pairing t-test), indicating that a more efficient introduction of indometacin is achieved after discrete bombardment with a low dose. As shown in Figure 4B, the skin damage was very minor at all sites and the Draize score was 1–2.

Hence, more efficient and improved drug introduction and less skin damage was achieved by controlling the bombardment dose. In addition, a suitable combination of helium pressure and bombardment dose can be used to deliver large microspheres incorporating drugs at different therapeutic doses to provide the desired pharmacological action.

Conclusion

We have demonstrated that the Helios gun system equipped with a cartridge container fitted with polyvinyl chloride resin as a rupture membrane allows successful quantitative and qualitative delivery of large microspheres of various size ranges (in particular 75–100 μm), in powder form, into hairless rat skin. This suggests a reduction in the barrier function of the stratum corneum, as far as drug introduction is concerned, and easy introduction of a variety of drugs with different physicochemical properties. Hence, water-soluble macromolecules, such as peptide and protein drugs, cytokines and vaccines, which are generally unstable in aqueous solution, as well as water-insoluble drugs, can be delivered into the skin.

The efficiency of introduction of indometacin (i.e. indometacin-loaded PLA microspheres) into the skin could be increased by choosing the appropriate helium pressure and particle size. More efficient and improved drug introduction and less skin damage was achieved by controlling the bombardment dose.

In conclusion, bombardment injection of drug-loaded microspheres by the Helios gun system has been shown to be a very useful tool for the delivery of a variety of powdered drugs and an alternative to parenteral injection via a needle, particularly for delivering water-soluble macromolecules.

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