

Effects of Particle Size, Helium Gas Pressure and Microparticle Dose on the Plasma Concentration of Indomethacin after Bombardment of Indomethacin-Loaded Poly-L-Lactic Acid Microspheres Using a HeliosTM Gun System

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We investigated the effects of the particle size of indomethacin-loaded poly-L-lactic acid microspheres (IDM-loaded PLA MS), the helium pressure used to accelerate the particles, and the bombardment dose of PLA MS on the plasma concentration of IDM after bombarding with IDM-loaded PLA MS of different particle size ranges, 20—38, 44—53 and 75—100 μm , the abdomen of hairless rats using the HeliosTM gene gun system (HeliosTM gun system). Using larger particles and a higher helium pressure, produced an increase in the plasma IDM concentration and the area under the plasma concentration–time curve (*AUC*) and resultant *F* (relative bioavailability with respect to intracutaneous injection) of IDM increased by an amount depending on the particle size and helium pressure. Although a reduction in the bombardment dose led to a decrease in C_{max} and *AUC*, *F* increased on decreasing the bombardment dose. In addition, a more efficient *F* was obtained after bombarding with IDM-loaded PLA MS of 75—100 μm in diameter at each low dose in different sites of the abdomen compared with that after bolus bombardment with a high dose (dose equivalent). These results suggest that the bombardment injection of drug-loaded microspheres by the HeliosTM gun system is a very useful tool for delivering a variety of drugs in powder form into the skin and systemic circulation.

Key words microparticulate bombardment system; HeliosTM gene gun system; microparticles

Recently, a microparticulate bombardment system has been investigated for the painless delivery of powdered drug through the stratum corneum to the epidermal–dermal interface using a high velocity supersonic flow of helium gas to accelerate the particles.¹⁾ One of the most attractive advantages of this approach is that the stratum corneum does not act as a rate-limiting barrier to drug penetration into the skin.²⁾ Several studies have shown successful local and systemic delivery of drugs, such as peptides, hormones, and vaccines, in animal models using a powder bombardment system, PowderJect.^{3–6)} We have also reported that the HeliosTM gene gun system (HeliosTM gun system) is a very useful tool for delivering a variety of drugs into the skin as well as introducing DNA and RNA into cells.⁷⁾ Although DNA- and RNA-coated gold and tungsten microparticles (1—3 μm in diameter and about 19 g/cm³ in density) loaded into a cartridge container of the HeliosTM gun system have been generally used for delivery into cells, indomethacin-loaded poly-L-lactic acid microspheres (IDM-loaded PLA MS) of different size ranges (20—38, 44—53 and 75—100 μm , about 1.2 g/cm³ in density) have been introduced quantitatively into hairless rat skin using the HeliosTM gun system equipped with a novel cartridge container and membrane that ruptures at a helium pressure of 100, 200 and 300 psi (1 psi = 6890 Pa).⁷⁾ Introduction of IDM-loaded PLA MS into the skin depended on the particle size and helium pressure, in that bombardment with the largest particles of diameter 75—100 μm at a pressure of 300 psi resulted in the highest amounts of IDM being introduced into the skin. In addition, the efficiency of IDM introduction after discrete bombardment with smaller amounts of microspheres of different sites of the abdomen of hairless rats increased significantly compared with that after a single bombardment with larger

amounts of microspheres. However, it is necessary to evaluate the systemic availability of IDM introduced into the skin by the HeliosTM gun system.

In the present study, we carried out a pharmacokinetic analysis to investigate the effects of particle size and bombardment dose of IDM-loaded PLA MS, and the helium pressure used to accelerate PLA MS on the plasma concentration of IDM after bombardment with IDM-loaded PLA MS using the HeliosTM gun system in hairless rats. We also evaluated the plasma IDM concentration after discrete bombardment.

MATERIALS AND METHODS

Materials Indomethacin was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Poly-L-lactic acid (Molecular weight *ca.* 10000) was obtained from Nacalai Tesque, Inc. (Kyoto, Japan). A polyvinyl chloride film (thickness 10 μm), used as the rupture membrane, was purchased from Hitachi Chemical Filtec Inc. (Tokyo, Japan). All other chemicals were of reagent grade.

Animals Male WBN/ILA-Ht hairless rats (weight 240—290 g) were supplied by the Life Science Research Center of Josai University (Saitama, Japan). The experiments were performed in accordance with the guidelines for animal use in the Life Science Research Center, Josai University.

Preparation of IDM-Loaded PLA MS IDM-loaded PLA MS were prepared as described previously.⁷⁾ Briefly, 100 mg IDM and 300 mg PLA were dissolved in 3 ml dichloromethane (CH_2Cl_2). This solution was then added to 150 ml 0.5% polyvinyl alcohol aqueous solution while stirring at 2000 rpm for 25 min at room temperature. Thereafter, the mixture was continuously stirred at 1000 rpm for 5 h at

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37 °C to evaporate CH₂Cl₂. The hardened IDM-loaded PLA MS were washed with distilled water then divided into the required particle size ranges using stainless-steel mesh sieves (20–38, 44–53 and 75–100 µm in diameter). The IDM content of PLA MS with a diameter of 20–38, 44–53 and 75–100 µm was 10.4, 18.8 and 22.2%, respectively.

Particle Bombardment of the Skin with IDM-Loaded PLA MS Using the Helios™ Gun System After anesthetizing (urethane 1 mg/kg i.p.) hairless rats, 0.5, 1.0 and 3.0 mg of IDM-loaded PLA MS (20–38, 44–53 and 75–100 µm in diameter) were transferred to the novel cartridge container fitted with a polyvinyl chloride film (thickness 10 µm) as a rupture membrane. The cartridge containers were placed in a cartridge holder with twelve holes. IDM-loaded PLA MS in the cartridge container was accelerated by the high velocity helium gas in the Helios™ gun system to bombard the shaved abdominal skin.⁷⁾ The helium pressures used were 100, 200 and 300 psi, respectively (1 psi=6890 Pa).

Discrete Particle Bombardment with IDM-Loaded PLA MS of Different Sites of the Skin Using the Helios™ Gun System After anesthetizing (urethane 1 mg/kg i.p.) hairless rats, 1.0 mg IDM-loaded PLA MS of 75–100 µm in diameter was transferred to each cartridge container. The IDM-loaded PLA MS in the cartridge container in a cartridge holder was then used to bombard the shaved abdominal skin at three different sites to deliver total amounts of 3.0 mg. The helium pressure used was 300 psi.

Intracutaneous (i.c.) Injection of IDM Solution After anesthetizing (urethane 1 mg/kg i.p.) hairless rats, 25 µl IDM (100 µg/kg in dose) in phosphate-buffered saline (PBS, pH 7.4) was injected intracutaneously into the abdomen.

Determination of Plasma Concentration of IDM Blood samples were collected from the jugular vein *via* a heparinized syringe at predetermined times and centrifuged at 13600 rpm (18000×*g*) for 5 min at 4 °C to obtain plasma. Plasma samples were mixed with acetonitrile containing *n*-hexyl *p*-hydroxybenzoate as an internal standard (1:2) and then centrifuged at 13600 rpm (18000×*g*) for 5 min at 4 °C. The obtained supernatants were stored in a refrigerator until analysis. IDM concentrations were determined by HPLC as described previously.⁷⁾

Data Analysis Plasma data were analyzed by a non-linear least squares regression program (Algorithm: Damping Gauss–Newton method).⁸⁾ The C_{\max} and T_{\max} values were obtained from the IDM concentration–time curves and the area under the plasma concentration–time curve (*AUC*) were calculated by the trapezoidal rule.

F_{0-24h} (relative bioavailability with respect to i.c. injection) was calculated as follows:

$$F_{0-24h} = \frac{AUC_{\text{bombard } 0-24h} / \text{Dose}_{\text{bombard}}}{AUC_{\text{i.c. } 0-24h} / \text{Dose}_{\text{i.c.}}} \times 100$$

where $AUC_{\text{bombard } 0-24h}$ and $AUC_{\text{i.c. } 0-24h}$ are the areas under the plasma concentration of IDM *versus* time (0–24 h) curves after particle bombardment and i.c. injection, respectively. $\text{Dose}_{\text{bombard}}$ and $\text{Dose}_{\text{i.c.}}$ are the IDM dose (µg/kg) in the particle bombardment and i.c. injection, respectively.

The absorption rate constant, k_a , and absorption profile of IDM after bombardment was obtained by applying a deconvolution method to the bombardment and i.c. data. The elim-

ination kinetic parameters for the i.c. injection were almost the same as those for the i.v. injection. The k_{12} , k_{21} , k_{el} , V_{ss} and total CL obtained from the i.c. data were 1.21, 0.530 and 0.0674 h⁻¹, and 190.0 ml/kg and 3.91 ml/h/kg, respectively. The maximum absorption rate (MAR) was obtained from the slope of the absorption profile. The slope of the profile was calculated for time points at 10 min intervals.

Statistical analyses were performed using Student's *t*-test.

RESULTS AND DISCUSSION

Effects of Particle Size, Helium Pressure and Bombardment Dose on the Plasma Concentration of IDM

Figure 1 shows typical plasma concentration–time curves of IDM after bombarding the abdomen of hairless rats with IDM-loaded PLA MS using the Helios™ gun system under various conditions. Table 1 summarizes the IDM pharmacokinetic parameters obtained. The efficiency of IDM introduction (% introduction) into the skin obtained previously is also listed.⁷⁾ The skin was excised immediately after bombardment of IDM-loaded PLA MS under different conditions and, thereafter, the amounts of IDM introduced into the skin were determined following extraction.

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

$$\% \text{ introduction} = \frac{\text{amount of IDM into the skin}}{\text{loading dose of IDM}} \times 100$$

where the loading dose of IDM is the IDM content of the PLA MS in the cartridge container. The recovery of IDM from skin lysate was almost complete ($99.8 \pm 0.66\%$, $n=6$).

When IDM-loaded PLA MS with different particle size ranges, at a dose of 1.0 mg, were bombarded at a helium pressure of 300 psi, the plasma concentration of IDM increased with increasing particle size (Fig. 1a). The C_{\max} and AUC_{0-24h} values increased in relation to the particle size (Table 1). The same trend was observed at helium pressures of 100 and 200 psi. The F_{0-24h} was also dependent on the particle size, although the C_{\max} and AUC_{0-24h} were affected by the MS drug content. As shown in Fig. 1b and Table 1, the plasma concentration of IDM after bombardment with IDM-loaded PLA MS of 75–100 µm in diameter, at the same dose of 1.0 mg, increased with increasing helium pressure. The C_{\max} , AUC_{0-24h} and F_{0-24h} values also increased in relation to the helium pressure. A helium pressure-dependence of F_{0-24h} was observed at all particle size ranges, in that a higher helium pressure produced a higher efficiency. The particle size and helium pressure were also found to correlate with the % introduction into the skin (Table 1). Consequently, good relationships between the F_{0-24h} and the % introduction, at each particle sizes for the helium pressure, were observed. Burkoth *et al.* have suggested that the release of drugs, introduced *via* the skin by the particle bombardment system, correlated well with results *in vivo*.³⁾ It seems, therefore, that the IDM plasma concentration depends on the amount of introduction, in that the F_{0-24h} is influenced by helium pressure used.

The absorption rate constant, k_a , was almost the same at a dose of 1.0 mg at the same helium pressure and particle size ranges, although the k_a s obtained at a helium pressure of 100 psi at each particle size range was different from those

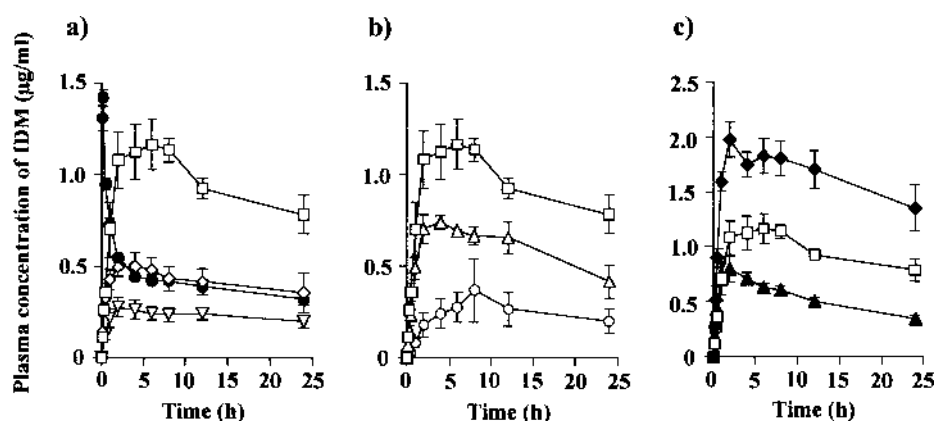


Fig. 1. Plasma Concentration of IDM after Bombardment with IDM-Loaded PLA MS Using the Helios™ Gun System under Various Conditions

a) Effect of particle size, b) effect of helium pressure, c) effect of bombardment dose. Needle injection: ●, i.c. injection (100 µg/kg). Particle bombardment: ▽, 20–38 µm, 1.0 mg, 300 psi; ◇, 44–53 µm, 1.0 mg, 300 psi; □, 75–100 µm, 1.0 mg, 300 psi; ○, 75–100 µm, 1.0 mg, 100 psi; △, 75–100 µm, 1.0 mg, 200 psi; ▲, 75–100 µm, 0.5 mg, 300 psi; ◆, 75–100 µm, 3.0 mg, 300 psi. Each data point represents the mean and the standard error ($n=3-6$).

Table 1. Percentage Introduction and Pharmacokinetic Parameters of IDM after Bombardment with IDM-Loaded PLA MS Using the Helios™ Gun System under Various Conditions

		% introduction	C_{\max} (µg/ml)	T_{\max} (h)	AUC_{0-24h} (µg·h/ml)	k_a (1/h)	MAR (µg/h)	$F_{0-24h}^{a)}$ (%)
i.c. IDM solution (100 µg/kg)	—	—	1.42 ± 0.05	0.17 ± 0.00	10.2 ± 0.38	—	—	—
20–38 µm ^{b)}	100 psi	20.7 ± 3.00	0.05 ± 0.01	12.0 ± 0.00	0.90 ± 0.10	0.171	1.45	2.20
1.0 mg MS/rat	200 psi	24.7 ± 4.11	0.21 ± 0.03	1.67 ± 0.33	3.58 ± 0.29	0.476	15.2	9.04
(104 µg IDM/rat)	300 psi	29.2 ± 4.20	0.28 ± 0.05	5.33 ± 3.33	5.49 ± 0.87	0.473	16.0	13.7
44–53 µm ^{b)}	100 psi	19.7 ± 2.65	0.13 ± 0.02	6.00 ± 1.54	2.41 ± 0.34	0.271	5.41	3.18
1.0 mg MS/rat	200 psi	25.5 ± 4.61	0.47 ± 0.06	4.00 ± 1.41	8.77 ± 1.32	0.496	27.3	11.7
(188 µg IDM/rat)	300 psi	30.1 ± 6.67	0.53 ± 0.07	2.67 ± 0.67	9.97 ± 1.66	0.499	37.4	13.7
75–100 µm								
0.5 mg MS/rat	300 psi	35.6 ± 4.16	0.81 ± 0.11	2.67 ± 0.67	12.5 ± 0.78	0.645	53.8	25.0
(111 µg IDM/rat)								
75–100 µm ^{b)}	100 psi	30.4 ± 6.00	0.39 ± 0.17	6.67 ± 0.67	5.81 ± 2.08	0.187	9.95	6.24
1.0 mg MS/rat	200 psi	33.8 ± 5.17	0.80 ± 0.04	6.00 ± 3.06	14.1 ± 1.01	0.361	39.6	14.7
(222 µg IDM/rat)	300 psi	39.6 ± 3.04	1.21 ± 0.12	6.00 ± 1.15	22.4 ± 2.08	0.346	56.0	22.9
75–100 µm								
3.0 mg MS/rat	300 psi	20.1 ± 1.73	1.97 ± 0.16	2.67 ± 0.67	38.8 ± 3.75	0.416	118	14.7
(666 µg IDM/rat)								

a) $F_{0-24h} = [(AUC_{\text{bombard } 0-24h} / \text{Dose}_{\text{bombard}}) / (AUC_{\text{i.c. } 0-24h} / \text{Dose}_{\text{i.c.}})] \times 100$. b) Relationship between the F_{0-24h} and the % introduction at each particle size range, 20–38 µm; $r=0.990$, 44–53 µm; $r=0.952$, 75–100 µm; $r=0.987$.

obtained at helium pressures of 200 and 300 psi (Table 1). In contrast, the MAR was dependent on the particle size and helium pressure under these conditions. Hence, the absorption rate of IDM (*i.e.* IDM absorption from the skin to systemic circulation) was found to being determined mainly by the amount of IDM introduced. These results indicate that bombardment with larger microspheres and at higher helium pressures used in this study leads to a more efficient introduction of IDM into the skin, thereby, allowing a higher F_{0-24h} for the IDM systemic availability.

When IDM-loaded PLA MS, 75–100 µm in diameter, at different doses were bombarded at a helium pressure of 300 psi, the plasma concentration of IDM increased with increasing bombardment dose (Fig. 1c). The C_{\max} and AUC_{0-24h} values also increased depending on the bombardment dose (Table 1). However, the calculated IDM F_{0-24h} increased on

decreasing the bombardment dose, in that a reduction in bombardment dose led to a higher efficiency of systemic absorption from the bombardment site. This is most likely due to a reduction in the particle collisions and in the coefficient of friction between the particles and the walls of the cartridge container after bombardment, thereby, allowing a higher particle velocity to produce a higher introduction and IDM F_{0-24h} . In addition, the larger microspheres used at lower doses tended to increase the IDM F_{0-24h} , particle size ranges of 75–100 µm generally being more efficient than those of 20–38 and 44–53 µm (data not shown). These results indicate that a more efficient IDM F_{0-24h} could be achieved by reducing the bombardment dose as well as the % introduction obtained. However, it should be noted that the plasma concentration of IDM decreased substantially on reducing the bombardment dose.

Effects of Discrete Bombardment of IDM-Loaded PLA MS on the Plasma Concentration of IDM In order to improve both the plasma concentration and F_{0-24h} after particle bombardment, IDM-loaded PLA MS, 75–100 μm in diameter, at a dose of 1.0 mg were used to bombard three different sites of the abdomen at a helium pressure of 300 psi to deliver total amounts of 3.0 mg as shown in Fig. 2 and Table 2. When each dose of 1.0 mg IDM-loaded PLA MS was used to bombard three different sites of the abdomen, the plasma concentration of IDM was much higher than that after bombardment with a bolus dose of 3.0 mg ($p < 0.01$). There was no significant difference between the F_{0-24h} values after discrete bombardment of each 1.0 mg dose and bolus bombardment of 1.0 mg, suggesting that discrete bombardment at different sites had no effect on the F_{0-24h} values achieved. In contrast, both the AUC_{0-24h} and F_{0-24h} values after discrete bombardment were about 1.5 times higher than those after bombardment with a bolus dose of 3.0 mg. Since the % introduction after bolus bombardment of 1.0 mg was about 2 times higher than that of 3.0 mg, the total amounts of IDM introduced after discrete bombardment of each 1.0 mg (dose

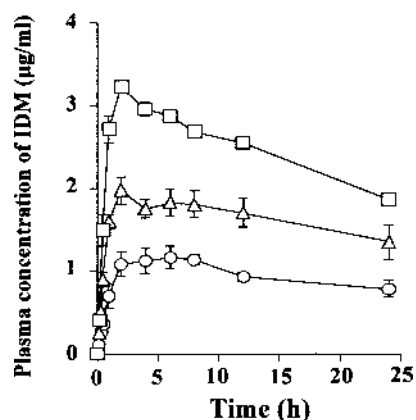


Fig. 2. Plasma Concentration of IDM after Bombardment with IDM-Loaded PLA MS Using the HeliosTM Gun System under Various Conditions

Particle size range, 75–100 μm . Helium pressure, 300 psi. Particle bombardment: ○, 1.0 mg MS in bolus; △, 3.0 mg MS in bolus; □, 1.0 mg MS×3 times. Each data point represents the mean and the standard error ($n=3-6$).

equivalent, 3.0 mg) were higher, thereby, allowing a higher F_{0-24h} and AUC_{0-24h} (i.e. amounts of IDM absorbed). Hence, we found that a more efficient F_{0-24h} was obtained after discrete bombardment at a low dose.

In the present study, we have demonstrated that a successful increase in the plasma IDM concentration is achieved after bombardment of IDM-loaded PLA MS with a larger particle size at a higher helium pressure using the HeliosTM gun system. In particular, a more efficient F_{0-24h} is obtained after discrete bombardment of PLA MS, 75–100 μm in diameter, at a low dose. Thus, a suitable combination of helium pressure and bombardment dose can be used for large microspheres incorporating drugs at different therapeutic doses to provide the desired pharmacological action.

In conclusion, the bombardment injection of drug-loaded microspheres by the HeliosTM gun system has been shown to be a very useful tool for the delivery of a variety of drugs in powder form into the skin and systemic circulation.

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Table 2. Percentage Introduction and Pharmacokinetic Parameters of IDM after Bombardment with IDM-Loaded PLA MS Using the HeliosTM Gun System under Various Conditions

	% introduction	C_{\max} ($\mu\text{g/ml}$)	T_{\max} (h)	AUC_{0-24h} ($\mu\text{g} \cdot \text{h/ml}$)	F_{0-24h}^a (%)
i.c. (100 μg IDM/kg)	—	1.42 ± 0.05	0.17 ± 0.00	10.2 ± 0.38	—
1.0 mg MS in bolus (111 μg IDM/rat)	39.6 ± 3.04	1.21 ± 0.12	6.00 ± 1.15	22.4 ± 2.08	22.9
3.0 mg MS in bolus (666 μg IDM/rat)	20.1 ± 1.73	1.97 ± 0.16	2.67 ± 0.67	38.8 ± 3.75	14.7
1.0 mg MS×3 times (666 μg IDM/rat)	38.4 ± 6.22	3.22 ± 0.24	2.00 ± 0.00	$58.9 \pm 4.10^*$	20.1

Particle size range, 75–100 μm . Helium pressure, 300 psi. a) $F_{0-24h}^a = [(AUC_{\text{bombard } 0-24h} / \text{Dose}_{\text{bombard}}) / (AUC_{\text{i.c. } 0-24h} / \text{Dose}_{\text{i.c.}})] \times 100$. * Significant differences, $p < 0.01$ compared with 3.0 mg MS in bolus for AUC_{0-24h} .