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Transdermal microparticle delivery by a supersonic-Helios^{TM} gun system

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The effect of particle size and flow of helium gas on the systemic absorption of indomethacin by means of a needle-free injection system was investigated. Poly-L-lactic acid microspheres conaining indomethacin were prepared by the o/w solvent evaporation technique. Microspheres were accelerated by a stream of helium gas in the Helios^{TM} gun system, and then delivered to the abdominal skin of male hairless rats. Delivery of indomethacin to rat skin proportionally increased with helium pressure (supersonic flow). Bioavailability and C_{max} were also dependent on the helium pressure. This method can be used to deliver the drug and/or microparticulate systems into the skin tissues and systemic circulation.

1. Introduction

Recently, a supersonic needle-free injection system has been introduced (Bellhouse et al. 1994; Burkoth et al. 1999). This system painlessly delivers drug powder through the stratum corneum to the epidermal-dermal interface using high velocity of supersonic flow of helium gas to accelerate the particles. One of the most attractive advantage in this system is that the stratum corneum does not work as a rate limiting-barrier for drug penetration in skin.

The Helios^{TM} gene gun system has been developed as a physical method to deliver gold particles containing DNA to plant and animal cells (Degano et al. 1998; Lai et al. 1995; Macklin et al. 1998; Saphie et al. 1996; Vanderzanden et al. 1998). This particle bombardment may also overcome the physical barrier to effective drug delivery, such as the stratum corneum.

In this study, we prepared poly-L-lactic acid microspheres (PLA MS) containing a model drug, indomethacin (IDM) and investigated the effect of particle size and supersonic flow of helium gas on the systemic absorption of IDM administered by the Helios^{TM} gun system.

2. Investigations, results and discussion

Scanning electron microphotograph shows IDM-loaded PLA MS with 75–100 μm in diameter. Table 1 summarizes mean particle size and IDM content of PLA MS with different size ranges.

Table 1: Mean particle size and IDM content of PLA MS with different size ranges

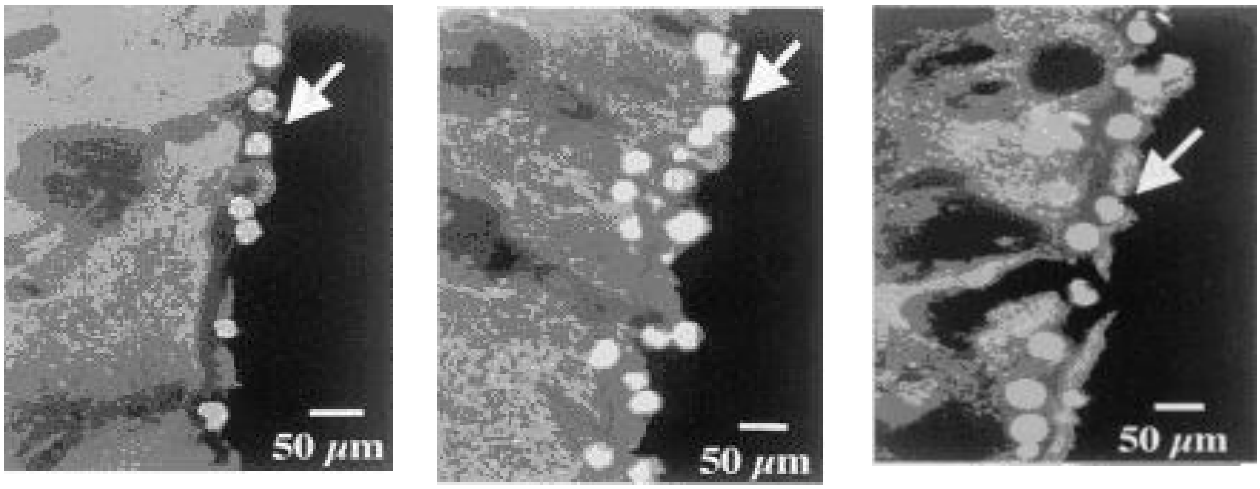
Size range (μm)	Mean particle size (μm)	IDM content (%)
20–38	30.3 ± 7.33	10.4
44–53	49.8 ± 6.41	18.8
75–100	92.2 ± 12.6	22.2

Volume-weighted mean ± S.D.

Table 2: Percent introduction and pharmacokinetic parameters of indomethacin after transdermal administration by the Helios^{TM} gun system

	Pressure (psi)	Introduction (%)	C_{max} (μg/ml)	T_{max} (h)	AUC_{0-24h} (μ · h/ml)	F^*_{0-24h} (%)
ic		–	1.42 ± 0.05	0.17 ± 0.00	10.2 ± 0.4	–
20–38 μm (1.7 mg/kg)**	100	10.6 ± 1.7	0.30 ± 0.15	14 ± 10	6 ± 3	3.58
	200	13.6 ± 0.9	1.0 ± 0.3	6.0 ± 2.0	16.1 ± 1.8	8.77
	300	13.9 ± 1.2	1.7 ± 0.4	7.3 ± 2.9	35 ± 7	18.4
44–53 μm (2.1 mg/kg)**	100	10.0 ± 1.8	0.19 ± 0.08	20 ± 4	3.8 ± 1.6	1.77
	200	13.6 ± 0.8	0.80 ± 0.09	10.7 ± 1.3	16.2 ± 1.7	7.84
	300	17.3 ± 1.8	2.0 ± 0.4	2.0 ± 0.0	37 ± 6	15.3
75–100 μm (2.3 mg/kg)**	100	10.3 ± 0.3	0.46 ± 0.14	7.3 ± 2.4	6.0 ± 1.4	2.43
	200	14.3 ± 1.8	1.39 ± 0.14	7.3 ± 2.9	29.0 ± 2.8	11.4
	300	20.1 ± 1.7	2.3 ± 0.3	3.3 ± 1.3	41 ± 7	17.9

*F_{0-24h}: [($AUC_{HeliosTM\ gun\ system}$/Dose)/9AUCic/Doseic]] × 100%; **indomethacin dose; Microspheres were administered 3.0 mg in dose(n = 3–4, mean ± SD)



a b c

Fig. 1: Confocal laser microphotographs of rat skin after transdermal administration of FITC (fluorescen isothiocyannate)-labeled polysty- rene MS (45 μm in mean diameter) by different pressures using

Fig. 1 shows confocal lazer microphoto graphs of the cross-section of hairless rat abdominal skin after bombardment of FITC-labeled polystyrene microspheres with 45 μm in mean diameter at different pressures (helium pressure: a: 100 psi, b: 200 psi c: 300 psi) using the Helios™ gun system.

Fig. 2 shows the relationship between helium pressure and percent introduction of IDM with the Helios™ gun system. Delivery of IDM to the hairless rat skin proportionally increased with helium pressure (supersonic flow). But there was no sufficient difference by particle size. High helium pressure is necessary to get a high efficacy of the IDM introduction. However, the skin turned red under high helium pressure (>300 psi).

Fig. 3 illustrates plasma IDM concentration-time curves after intracutaneous administration of the different sieved PLA MS containing IDM. Table 2 summarizes the pharmacokinetic parameters. Plasma concentration of IDM increased with increasing helium pressure in both cases. These results were correspondent to IDM delivery to skin. Moreover, plasma concentration was maintained in all cases at least until 12 h after administration. Bioavailability and C_{max} were also dependent on helium pressure.

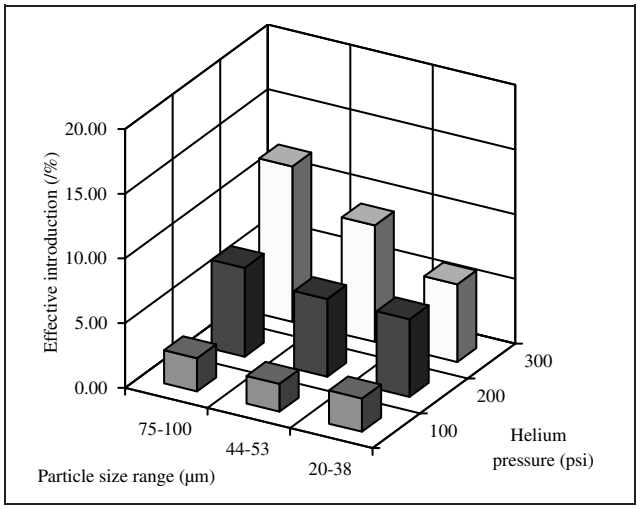


Fig. 2: Relationship between helium pressure, particle size and effective introducton (mean, n = 3–4)

3. Experimental

3.1. Materials

Indomethacin was purchased from Wako Pure Chemical (Osaka, Japan). PLA was purchased from Ogi Chemical Co. (Hyogo, Japan). The Helios™ gun system was purchased from Bio-Rad Laboratories, Richmond (CA,USA). FITC (fluorescent isothiocyanate)-labeled polystyrene microspheres (45 μm in diameter) were purchased from Polysciences Inc. (Warrington, PA, USA). Confocal laser microphotographs, MRC-600 Lasersharp System was purchased from Bio-Rad Laboratories, Richmond, CA (USA). Tissue– Teck was purchased from Sakura Finetechnical Co., Ltd. (Tokyo, Japan).

3.2. Preparation of PLA MS containing IDM

PLA MS containing IDM were prepared by the oil/water (o/w) solvent evaporation technique (Qiu et al. 1996). PLA (MW 10 kDa, 300 mg) and IDM (100 mg) dissolved in cold CH_2Cl_2 (about 3 ml) were added in cold 0.5% poly-vinyl alcohol (PVA) aqueous solution (150 ml) while stirring at 2,500 rpm. After stirring for 25 min at 2,000 rpm, the solvent was continuously evaporated by stirring at 1,000 rpm at 37 °C. The hardened PLA MS was filtered and washed with distilled water. After drying, PLA MS was sieved into the required particle size ranges (44–53 and 75–100 μm) using stainless steel mesh sieves. The IDM content in PLA MS was determined to about 20% by an extraction method. The IDM content in the microspheres were assayed by HPLC.

3.3. Packing of PLA MS in tube cartridge of Helios™ gun system

Three milligrams of sieved PLA MS containing IDM was filled in an stainless steel tube cartridge having a membrane sheet made of poly-vinyl chloride at the exit side. This cartridge was inserted into the cartridge holder of the Helios™ gun system.

3.4. Introduction of IDM in skin

After anesthetizing the male hairless rats (240–290 g), PLA MS containing IDM filled in the tube cartridge were accelerated by streams of helium gas of various velocity (100, 200 and 300 psi) in the Helios™ gun system. The treated abdominal skin (5 cm^2) was then excised immediately after treatment, and the skin surface was washed and cleaned by physiological saline and gauze. The skin was homogenized with 5 ml of acetone : acetonitrile (1 : 5) and shaken for 5 min. After centrifugation, the organic phase (0.5 ml) was collected and 0.5 ml of internal standard solution was added. Samples were stocked in a refrigerator until analysis.

3.5. Systemic IDM delivery

The sieved PLA MS containing IDM were delivered to the abdomen of hairless rats in the same manner as described above. Plasma samples (0.1 ml) were collected from the jugular vein at predetermined times. The sample was diluted twice by PBS and acetonitrile (0.2 ml) containing internal standard was added to the mixture. After shaking and centrifugation at 13600 rpm, the obtained supernatant was collected and stocked in a refrigerator unitil analysis. The supernatant (200 μl) was applied to HPLC twice.

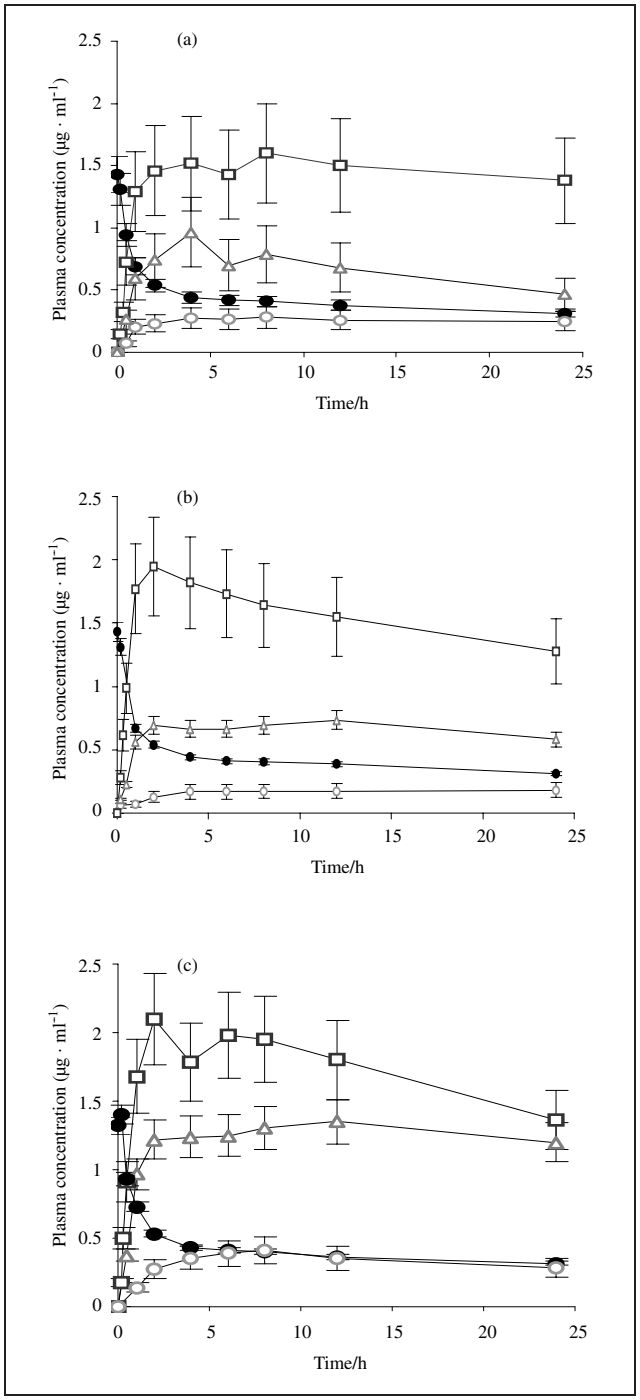


Fig. 3: Plasma concentration of indomethacin after transdermal administration of PLA MS containing indomethacin by Helios gun system (a) 20–38 μm in diameter, (b) 44–53 μm in diameter, (c) 75–100 μm in diameter l; i.c. injection (0.1 mg/kg), ○; 100 psi, △; 200 psi, □; 300 psi (mean ± S.E., n = 3–4)

3.6. Sample analysis

IDM concentration in the obtained samples was determined by HPLC. The equipment was composed of a Shimadzu LC-6A pump, a Shimadzu SPD-6A UV detector, aq Rheodyne 7125 injector, and a reversed-phase column (Inertsil ODS-2, 5 μm, 4.6 × 250 mm). For IDM analysis, the UV detector was operated at 262 nm, and a mobile phase containing 45% water, 55% acetonitrile, and 0.1% phosphoric acid flew at 1.2 ml/min.

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