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RESEARCH PAPER

Preclinical Pharmacokinetics and Biodistribution of Human Papillomavirus DNA Vaccine Delivered in Human Endogenous Retrovirus Envelope-Coated Baculovirus Vector

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ABSTRACT

Purpose Test pharmacokinetics and biodistribution of a human papillomavirus(HPV)16L1 DNA vaccine delivered in human endogenous retrovirus envelope protein (HERV)-expressing baculovirus (AcHERV) and those of naked plasmid vaccine.

Method HPV16L1 gene was administrated as a naked plasmid or in AcHERV to mice via intravenous and intramuscular routes. HPV16L1 gene was extracted and assayed by quantitative real-time polymerase chain reaction, which was determined to have a detection limit of 50 copies/μg genomic DNA..

Results Mean residence times of HPV16L1 in AcHERV were 4.8- and 272.2-fold higher than naked HPV16L1 DNA vaccines after intramuscular and intravenous administration, respectively. Naked HPV16L1 DNA levels 1 month after injection were >3 orders of magnitude lower in each tissue tested than AcHERV-delivered HPV16L1, which was retained in most tissues without specific tissue tropism. AcHERV-delivered HPV16L1 administered intramuscularly persisted at the injection sites. However, the levels of copy numbers in muscle were low (1,800/μg genomic DNA) after 1 month, and undetectable after 6 months.

Conclusions HPV16L1 delivered via AcHERV resides longer in the body than HPV16L1 in naked form. The lack of tissue tropism ensures the safety of AcHERV vectors for further development.

KEY WORDS biodistribution · DNA vaccine · human endogenous retrovirus envelope-expressing baculoviral vector · human papillomavirus · pharmacokinetics

ABBREVIATIONS

AcHERV	human endogenous retrovirus envelope protein-expressing baculovirus
AUC	area under the curve
AUMC	area under the momentum curve
CV	coefficient of variation
GAPDH	glyceraldehyde 3-phosphate dehydrogenase
gDNA	genomic DNA
HERV	human endogenous retrovirus envelope protein
HPV	human papillomavirus
MRT	mean residence time
QRT-PCR	quantitative real-time polymerase chain reaction
VLP	virus-like particles

INTRODUCTION

Human papillomavirus (HPV) infection is the main factor associated with the development of cervical cancer—the third

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leading cause of cancer death in women (1,2). HPV infection is preventable by prophylactic vaccination with virus-like particles (VLPs). Furthermore, VLPs induce high levels of neutralizing antibodies in immunized animals without an adjuvant. Two HPV VLP vaccines are available to prevent infection by high-risk HPV types: Gardasil marketed by Merck, and Cervarix, marketed by Glaxo–Smith–Kline. However, there still exists a need for more effective vaccines (3,4).

Non-replicating viral vectors have recently been studied as a means to enhance the delivery efficiencies of plasmid DNA vaccines. Non-replicating viral vectors are advantageous in that they eliminate the safety concerns associated with replicating viral vectors. Wu *et al.* (5) reported the use of a non-replicating papillomavirus pseudovirion to increase the delivery efficiency of DNA vaccines. Our group recently developed non-replicating baculovirus vector-based HPV DNA vaccines to enhance cellular delivery of HPV16L1-encoding genes (6,7). These recombinant baculovirus vectors were genetically engineered to bear human endogenous retrovirus envelope protein (HERV) on the surface to enhance the intracellular delivery of HPV16L1 DNA via endogenous retrovirus receptor-mediated endocytosis. The HERV-expressing baculoviral vectors (AcHERV) have the advantage of being non-replicating in mammals; thus, they reduce the potential adverse effects associated with replicating viral vectors, such as conversion to pathogenic strains. After intramuscular injection, HERV-expressing baculoviral HPV DNA vaccines were shown to induce both cell-mediated immunity and humoral immune responses to HPV16L1, provoking cellular immune responses greater than Gardasil (8).

Pharmacokinetic and biodistribution studies of the recombinant DNA vaccine are essential for the preclinical development of AcHERV-based HPV16L1 vaccines. Because AcHERV vectors are newly constructed for use as DNA vaccine delivery vectors, the pharmacokinetics and distribution behaviors of DNA vaccines administered in AcHERV vector systems are incompletely understood. In this study, we tested whether the pharmacokinetics and biodistribution patterns of HPV16L1 DNA vaccine delivered in AcHERV differed from those of naked HPV16L1 plasmid vaccines after administration via intramuscular and intravenous routes.

MATERIALS AND METHODS

Preparation of DNA Vaccines

HPV16L1-encoding DNA vaccines were constructed as previously described (8). Briefly, the HPV16L1 gene was cloned into pFastBacTM1 (Invitrogen, Carlsbad, CA, USA) to yield pFB-HPV16L1. A synthetic, codon-optimized envelope gene of HERV type W (GenBank accession number NM014590; GenScript Corp., Piscataway, NJ, USA) was then inserted into pFB-HPV16 L1. The resulting plasmid, termed pFB-HERV-HPV16L1, was purified using a Qiagen Mega prep kit (Qiagen, Hilden, Germany) to yield an endotoxin-free plasmid. The recombinant baculovirus AcHERV-HPV16L1 expressing the HERV envelope protein and HPV16L1 was produced from pFB-HERV-HPV16L1 using the Bac-to-Bac Baculovirus Expression System (Invitrogen), according to the manufacturer's instructions. A schematic diagram of the DNA vaccines is shown in Fig. 1. The corresponding copy number was calculated using the following equation (9,10):

$$\mathrm{DNA(copy)} = \left[\left(6.02 \times 10^{23}\mathrm{copy/mol}\right) \times \mathrm{DNA\ amount(g)}\right] / \left[\mathrm{DNA\ length(bp)} \times 660\mathrm{(g/mol/bp)}\right]$$

Vaccine Administration

Six-week-old female BALB/c mice, purchased from Daehan Biolink (Seungnam, Korea), were housed under filter-top conditions in a Bio-safety Level 2 facility and provided free access to food and water during the experiments. All animals were maintained and used in accordance with the Guidelines for the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, Seoul National University. Recombinant baculoviruses were diluted in sterile phosphate-buffered saline (PBS) to 1×10^7 copies/50 μl. At time-point zero, mice were immunized by intramuscular injection into the hind leg of 1×10^7 or 1×10^{13} copies (approximately 100 μg) of pFB-HERV-HPV16L1, or 1×10^7 copies of AcHERV-HPV16L1 in a total injection volume of 50 μl.

Evaluation of HPV16L1 Expression in Muscle Tissues by Immunoblotting

The expression of HPV16L1 proteins in muscle tissues was verified by immunoblotting. Mice were intramuscularly administered with 1×10^7 copies of pFB-HERV-HPV16L1, 1×10^{13} copies of pFB-HERV-HPV16L1 or 1×10^7 copies of AcHERV-HPV16L1, respectively. Two days after dosing animals, muscle tissues at the injection sites were sampled and lysed by incubating for 10 min at 4°C in cell lysis buffer (20 mM Tris-HCl, 150 mM NaCl, 1 mM disodium-EDTA, 1 mM β-glycerophosphate, 1 mM Na_3Vo_4, 2.5 mM sodium pyrophosphate). The cell debris was removed by centrifugation at 13,000 rpm at 4°C for 10 min. The proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 12% gels, and then electrotransferred to a nitrocellulose membrane in transfer buffer (50 mM Tris-glycine pH 9.0, 20% methanol). The membrane was blocked and incubated with an anti-HPV16L1 CamVir-1 monoclonal antibody

(1:1000; RDI, Boston, MA, USA) or a β-actin antibody (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA). After several washings, the membrane was incubated with alkaline phosphatase-conjugated anti-mouse IgG antibody (1:2000; Santa Cruz Biotechnology). The membrane was incubated with alkaline phosphatase buffer containing 0.33% bromochloroindolyl phosphate and 0.165% nitroblue tetrazolium until color developed.

Blood and Tissue Collection

Tail vein blood samples were obtained at various time points after administration using capillary tubes. For biodistribution studies, different groups of mice were sacrificed by CO_2 inhalation at various time points after administration of DNA vaccines, and tissues were collected and stored at -80°C for subsequent analysis. Total tissue DNA was purified using a DNeasy Tissue Kit (Qiagen), as described by the manufacturer. For assessment of DNA persistence at the injection site, mice were sacrificed and muscle tissues were collected 15 min, and 1, 15, 30 days, 6 months, and 12 months after administration of DNA vaccines.

Pharmaokinetic Data Analysis

Pharmacokinetic parameters were analyzed using the Winnonlin program (Pharsight, St. Louis, MO, USA). Area under the curve (AUC), and area under the momentum curve (AUMC) were calculated by extrapolating the data to infinity. The absolute bioavailability values of naked and baculoviral DNA vaccines after intramuscular administration were calculated by dividing AUC after intramuscular administration with that after intravenous administration. Mean residence time (MRT) was determined by non-compartmental analysis.

Quantitative Real-time Polymerase Chain Reaction (QRT-PCR) Detection of HPV16L1 DNA

The linearity, copy number detection limit, and R^2 of QRT-PCR analyses were validated by running standards in parallel with or without gDNA samples of various tissues obtained from control mice. Ten-fold serial dilutions of pFB-HERV-HPV16L1, ranging from 1×10^1 to 1×10^8 copies/μl, were used to construct the standard curves. Levels of the HPV16L1 gene in mouse gDNA samples were determined using the validated QRT-PCR assay. A gDNA template (200 ng) was amplified using LightCycler FastStart DNA Master SYBR Green I (Roche Diagnostics Gmbh, Mannheim, Germany). The amplification conditions consisted of an initial denaturation step at 94°C for 7 min, and 30 cycles of 30 s at 94°C, 20 s at 62°C, and 20 s at 72°C. The primers for HPV16L1 detection were 5′-CAG CGA GAC CAC CTA CAA GA-3′ (forward primer) and 5′-GCT GTT CAT GCT GTG GAT GT-3′ (reverse primer), generating a 139-bp product. Amplification of glyceraldehyde phosphate dehydrogenase (GAPDH) mRNA was used to control for the efficiency of QRT-PCR among samples. The coefficient of variation (CV), a measure of reliability and reproducibility (11), was calculated using the following equation:

$$\mathrm{CV} = (\text{standard deviation of reference gene}/\text{average of reference gene}) \times 100\%$$

Statistics

Statistical analyses of data were conducted using analysis of variance (ANOVA). A P-value less than 0.05 was considered significant. The Student–Newman–Keuls method was used as a post hoc test. Statistical analyses were conducted using SigmaStat v.3.5 (Systat Software, Richmond, CA, USA).

RESULTS

Efficient Expression of HPV16L1 Protein After Delivery in AcHERV

The construction scheme of the HPV16L1 DNA vaccine in AcHERV is described in Fig. 1. First, we compared the HPV16L1 protein expression levels at the injection site after administration of HPV16L1 in naked plasmid DNA (pFB-HERV-HPV16L1) and AcHERV forms. The expression efficiency of HPV16L1 at the injection site was notably higher following administration of the HPV16L1 gene in AcHERV compared to that of the naked plasmid DNA form. When HPV16L1 was administered as 1×10^7 copies in the naked plasmid form, HPV16L1 protein was not detected 2 days post-injection (Fig. 2). In contrast, the administration of HPV16L1 in AcHERV at the same L1 copy number (1×10^7 copies) resulted in clear expression of HPV16L1 protein (Fig. 2). Similar levels of HPV16L1 protein were obtained when the injection dose of naked plasmid HPV16L1 DNA vaccine was increased to 1×10^{13} copies. Based on these expression results, we used an injection dose of naked plasmid DNA vaccine of 1×10^{13} copies for further pharmacokinetic and biodistribution studies.

Validation of QRT-PCR

To quantify the levels of DNA vaccines in biological samples, we used QRT-PCR. The QRT-PCR method was first validated with respect to sensitivity (quantification limit), specificity, precision, and linearity in the detection of the HPV16L1 gene. Ten-fold serial dilu-

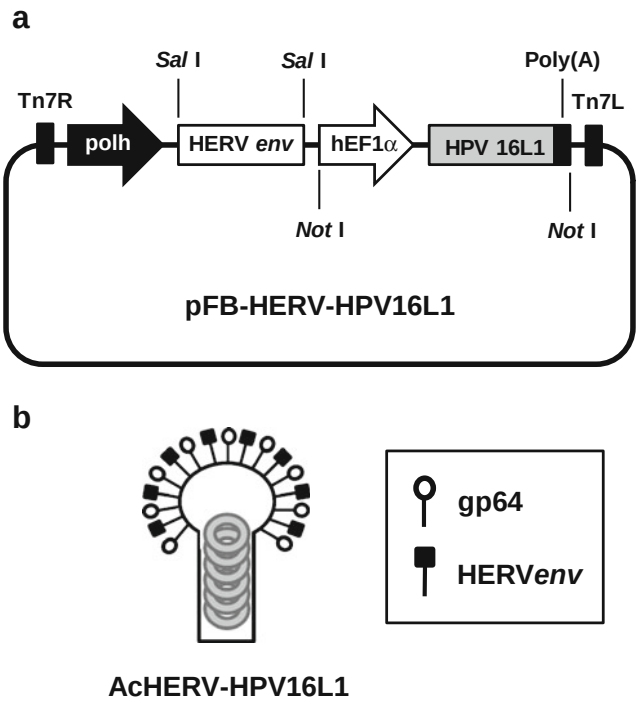

Fig. 1 Schematic diagram of HPV16L1 DNA vaccines. (**a**) The pFB-HERV-HPV16L1 plasmid was constructed for naked HPV16L1 DNA vaccines. (**b**) The AcHERV-HPV16L1 DNA vaccine was constructed to deliver HPV16L1 DNA in a HERV-expressing baculoviral vector.

tions of pFB-HERV-HPV16L1, ranging from 1×10^1 to 1×10^8 copies/μl, were used to construct standard curves in a background of 200 ng genomic DNA (gDNA) derived from 11 different tissues, including muscle. Linear regression analyses of the standard curves produced slopes less than -2.86 and greater than -3.25, and correlation coefficients (R^2) greater than 0.98 (Table I). The detection limit was determined to be 10 copies (approximately 100 ag plasmid DNA) in 200 ng gDNA from each tissue. For confirmation of absolute quantification using 200 ng gDNA as a normalizing parameter, the GAPDH gene was amplified within a background of 200 ng gDNA from each tissue. In all cases, the CV values of the absolute quantification were less than 4%, ranging from 0.4% to 3.1% (Table I).

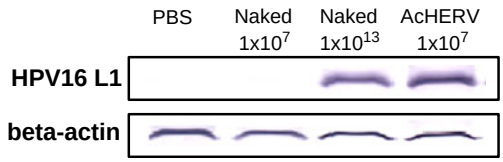

Fig. 2 Expression of HPV16L1 protein at the injection site. Mice were intramuscularly injected with PBS, 1×10^7 copies of pFB-HERV-HPV16L1, 1×10^{13} copies of pFB-HERV-HPV16L1, or 1×10^7 copies of AcHERV-HPV16L1. The expression of HPV16L1 proteins in muscle tissues was analyzed by immunoblotting 2 days after injection.

Pharmacokinetics of HPV16L1 DNA Vaccine Administered in AcHERV

The pharmacokinetic profile of the HPV16L1 DNA vaccine in AcHERV was significantly different from that of the HPV16L1 DNA vaccine in the naked plasmid form. Following intravenous administration, the concentration of naked HPV16L1 DNA vaccines in the blood decreased by more than five orders of magnitude in 24 h (Fig. 3a). In contrast, the blood level of HPV16L1 DNA vaccine in AcHERV was only one order of magnitude lower 24 h after intravenous administration. Following intramuscular administration of the naked plasmid form, HPV16L1 levels peaked at 6 h and decreased thereafter (Fig. 3b). In the case of HPV16L1 DNA in AcHERV, the blood concentrations peaked at 1.5 h. However, the blood levels at 3 h did not significantly differ from those at 24 h. Thirty days after a single intravenous injection of HPV16L1 in naked plasmids, blood levels had decreased by more than seven orders of magnitude compared to the blood levels at 15 min (Fig. 3c). In contrast, the blood levels of HPV16L1 delivered in AcHERV 30 days after administration were only two orders of magnitude lower than those at 15 min. Thirty days following intramuscular administration, the blood levels were 120- and 20-fold lower than those at 15 min for the naked form and AcHERV form, respectively (Fig. 3d). Pharmacokinetic parameters support the difference in the profiles of HPV16L1 DNA in naked plasmids and in AcHERV (Table I). The MRT of AcHERV-HPV16L1 was 4.8- and 272.2-fold higher than that of pFB-HERV-HPV16L1

Table I Validation of the QRT-PCR Method in Each Tissue

Tissue type	Slope	R^2	Ct (mean ± SD)[a]	CV (%)
Muscle	−3.25	0.99	10.51 ± 0.33	3.1
Blood	−3.25	0.99	10.41 ± 0.18	1.7
Thymus	−2.93	0.99	10.25 ± 0.14	1.3
Lymph node	−3.13	0.98	10.32 ± 0.13	1.2
Spleen	−3.15	0.98	9.75 ± 0.14	1.4
Liver	−2.88	0.98	10.02 ± 0.05	0.5
Lung	−3.14	0.98	10.12 ± 0.04	0.4
Kidney	−2.94	0.98	10.11 ± 0.17	1.7
Heart	−3.00	0.98	9.91 ± 0.08	0.8
Brain	−2.86	0.98	9.73 ± 0.21	2.2
Ovary	−3.01	0.98	10.08 ± 0.17	1.7

Ten-fold serial dilutions of pFB-HERV-HPV16L1, ranging from 1×10^1 to 1×10^8 copies/μl, were used to construct the standard curves in solutions containing 200 ng gDNA from each tissue.

[a] The amplification of GAPDH in solutions containing 200 ng gDNA from each tissue ($n=6$)

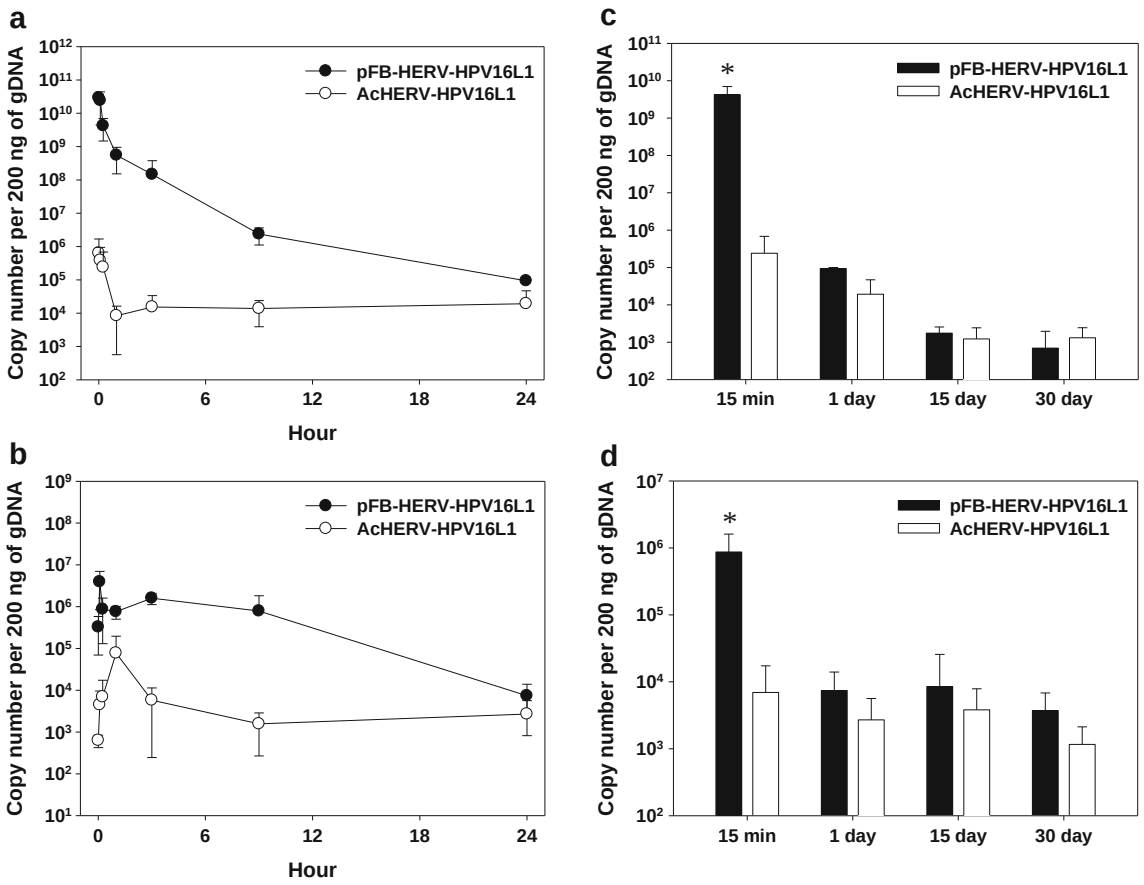

Fig. 3 Time-dependent blood concentration profiles of HPV16L1 DNA vaccines. Mice were injected with 1×10^{13} copies of pFB-HERV-HPV16L1 or 1×10^{7} copies of AcHERV-HPV16L1 via intravenous (**a**, **c**) or intramuscular (**b**, **d**) routes. After single injection, blood samples were collected until 30 days (**c**, **d**). The HPV16L1 copy number in blood was determined by QRT-PCR. Results are expressed as the mean ± S.D ($n = 5$).

after intramuscular and intravenous administration, respectively (Table II).

Persistence at the Injection Site

The rate of clearance of DNA vaccine differed between the naked plasmid DNA vaccine and the AcHERV-based HPV DNA vaccine after intramuscular administration in mice (Fig. 4). The AcHERV-based DNA vaccine showed much slower clearance rates at the injection site than the naked plasmid DNA vaccine. One day after administration of HPV16L1 in naked plasmids, the number of HPV16L1 gene copies remaining in muscle decreased 1.5×10^{5}-fold relative to the levels at 15 min (Fig. 4a). One day after intramuscular administration of AcHERV-HPV16L1, the number of HPV16L1 gene copies remaining in muscle decreased 2.2×10^{2}-fold compared to that at 15 min (Fig. 4a). Expressed as a percentage of the levels 15 min after injection, HPV16L1 gene copies in muscle 30 days after injection of the naked form and AcHERV-HPV16L1 were 0.0003% and 0.04%, respectively. At 6 months after injection, complete clearance of HPV16L1 gene in muscle was observed in mice treated with naked plasmid DNA orAcHERV-HPV16L1 (Fig. 4a).

Notably, the initial distribution from muscle to blood was much higher after intramuscular injection of HPV16L1 in AcHERV than in the naked form: 15 min post administration, the blood-to-muscle ratios were 881.9-fold higher in the AcHERV-treated group than in the naked plasmid DNA-treated group (Fig. 4b). The blood-to-muscle ratios were determined only up to 1 month, since there was no detection of HPV16L1 gene in muscle at 6 month time point.

Biodistribution of DNA Vaccines

The distribution profiles of naked plasmid DNA and AcHERV-based HPV16 DNA vaccines after intramuscular administration in mice were different (Fig. 5). Compared to the naked plasmid form, AcHERV-based HPV16L1 DNA vaccines showed greater persistence not only at the injection site but also in other organs. Fifteen minutes after

Table II Pharmacokinetic Parameters After Administration of HPV16L1 DNA in Naked Plasmid DNA and in AcHERV

	pFB-HERV-HPV16L1[a]		AcHERV-HPV16L1[b]	
	IM	IV	IM	IV
Tmax (hr)	0.08 ± 0.01	0.00 ± 0.01	1.00 ± 0.05	0.00 ± 0.00
Cmax ($mg \cdot ml^{-1}$)	2.50 ± 0.53	19033.28 ± 1602.70	0.75 ± 0.20	6.49 ± 2.40
$t_{1/2}$ (hr)	87.66 ± 9.70	99.27 ± 6.19	289.13 ± 12.22	174.27 ± 11.21
Absolute bioavailability	0.0003 ± 0.0002	1.00 ± 0.00	0.55 ± 0.16	1.00 ± 0.00
AUC_∞ ($mg \cdot ml^{-1} \cdot hr$)	14.02 ± 0.82	4839.33 ± 541.41	25.63 ± 6.88	46.31 ± 6.90
$AUMC_\infty$ ($mg \cdot ml^{-1} \cdot hr^2$)	1320.64 ± 248.63	2722.58 ± 297.97	11538.51 ± 2167.50	7058.80 ± 1594.85
MRT_∞ (hr)	94.21 ± 16.14	0.56 ± 0.01	450.18 ± 11.87	152.43 ± 20.25

[a] Mice were injected with 1×10^{13} copies (101.9 μg) of pFB-HERV-HPV16L1

[b] Mice were injected with 1×10^{7} copies (1.55 ng) of AcHERV-HPV16L1

injecting naked pFB-HERV-HPV16L1 plasmids, HPV16L1 gene levels were 16-, 2-, and 8-fold higher in the lymph node, liver, and ovary tissue, respectively, than in blood (Fig. 5a). However, 1 month after injection with pFB-HERV-HPV16L1, the HPV16L1 gene was detected mainly in muscle, and was significantly decreased in other organ tissues, typically to levels less than 520 copies per 200 nanograms of gDNA. Six months after injection with pFB-HERV-HPV16L1, the HPV16L1 gene was not detectable at muscle, blood, and thymus. In other tissues, the levels of HPV16L1 gene were less than 100 copies per 200 nanograms of gDNA.

In contrast to the distribution profiles of naked plasmid DNA, animals intramuscularly injected with AcHERV-HPV16L1 retained most L1 gene copies in most organ tissues after 1 month; the exception was muscle tissue, where the HPV16L1 gene was decreased 2547.7-fold at 1 month (Fig. 5b). One month after injection of AcHERV-HPV16L1, the mean levels of the HPV16L1 gene in the lymph node and lung tissues, though 3.4- and 2.9-fold higher, respectively, compared to those at 15 min, were not significantly different (p-values of 0.053 and 0.192, respectively). Six months after injection of AcHERV-HPV16L1, the HPV16L1 gene was not detectable at muscle, blood, thymus, and lung. In other tissues, the levels of HPV16L1 gene were less than 200 copies per 200 nanograms of gDNA. Twelve months after injection of AcHERV-HPV16L1(Fig. 5b), the HPV16L1 gene was not detectable at all tissues tested.

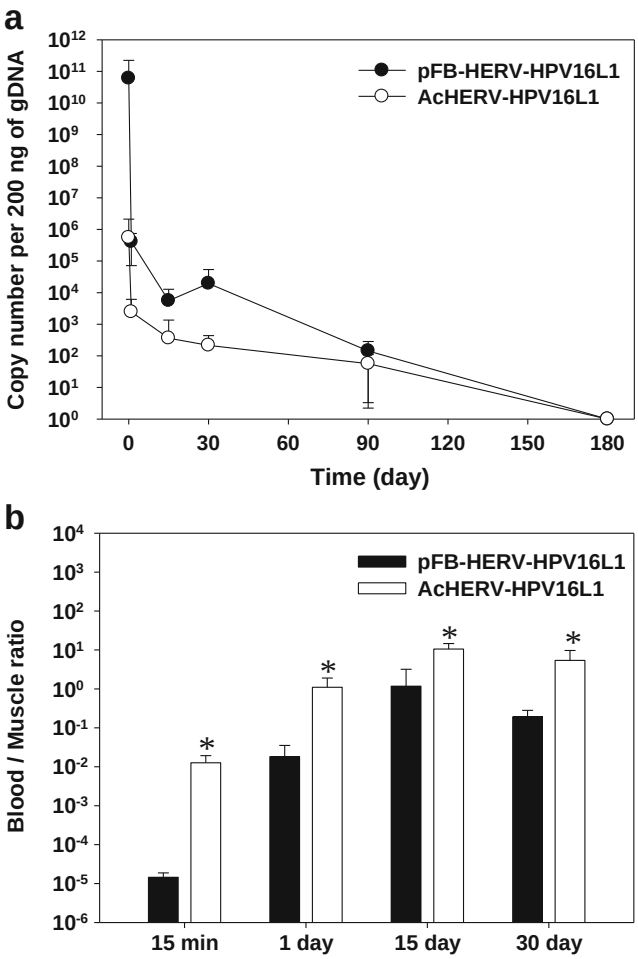

Fig. 4 Persistence of the DNA vaccines at the injection site. (**a**) HPV16L1 copy numbers in muscle were determined by QRT-PCR after intramuscular administration of HPV16L1 in naked form and in AcHERV. (**b**) Clearance from muscle to blood was analyzed based on the blood-to-muscle ratio. Results are expressed as the mean ± S.D (*$p < 0.05$ compared to the naked form; $n = 5$).

DISCUSSION

In this study, we reported the pharmacokinetic and biodistribution profiles of AcHERV-based DNA vaccines in mice. QRT-PCR was validated and used to perform pharmacokinetic and biodistribution studies of the

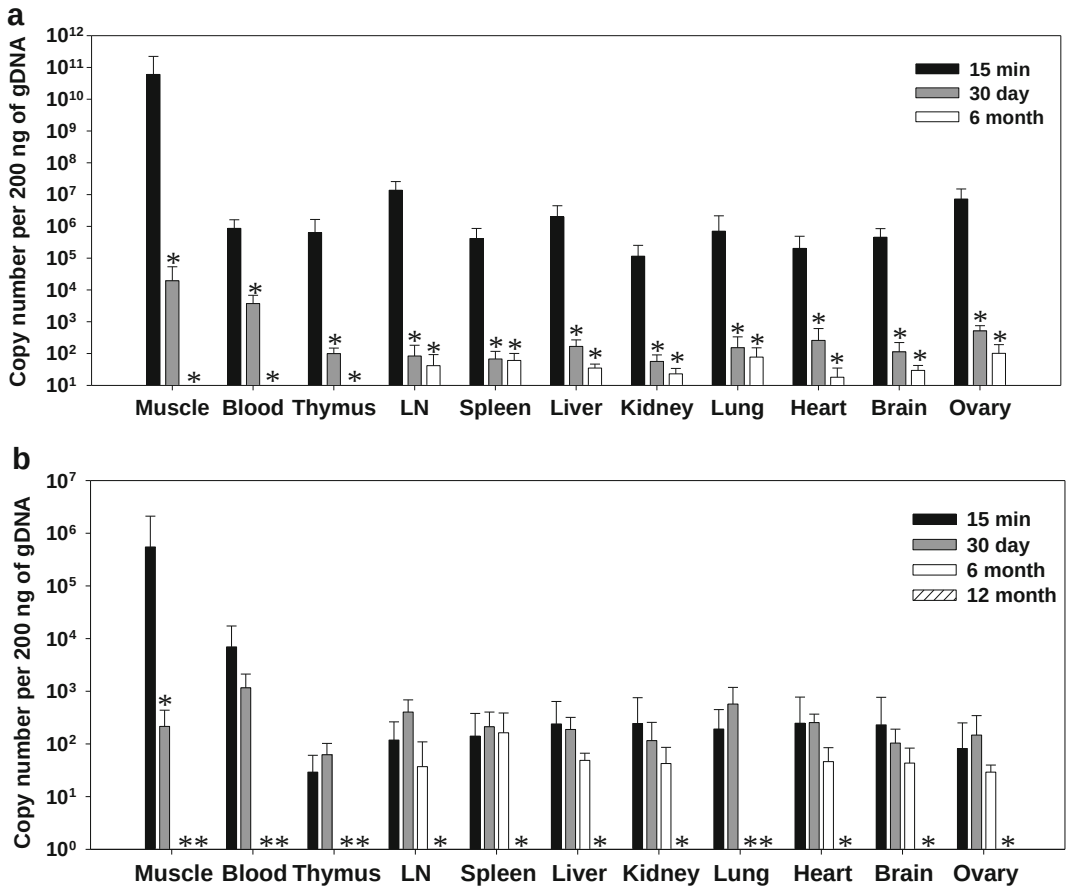

Fig. 5 Organ distribution of the DNA vaccines. HPV16L1 copy number was determined in various organ tissues after intramuscular administration in the naked plasmid form (**a**) and in AcHERV (**b**). HPV16L1 copy numbers per 200 nanograms gDNA were measured at various time points after injection. Results are expressed as the mean ± S.D (*$p < 0.05$ compared to the 15-minute point; $n = 5$).

HPV16L1 gene administered in naked plasmid DNA and in AcHERV. Compared to naked plasmid DNA encoding HPV16 L1, the AcHERV-HPV16L1 DNA vaccines showed prolonged retention *in vivo*.

We observed that HPV16L1 in AcHERV provided more effective antigen expression at the injection site compared to HPV16L1 in naked plasmids. The higher expression of HPV16L1 in AcHERV might be due to increased cellular uptake of AcHERV vectors. Indeed, in our previous study (8), we observed higher expression of HPV16L1 in murine cell lines following delivery in AcHERV than in plasmid DNA. Although AcHERV may have greater affinity for human cells than murine cells, the higher expression of antigen proteins in murine muscle tissues supports the use of mice as a suitable animal model for pharmacokinetic and biodistribution studies.

To quantify HPV16L1 DNA in blood and tissue samples, we used a validated QRT-PCR approach. A classical pharmacokinetic analysis using liquid chromatography is not suitable for DNA-based drugs owing to the large molecular weight of these agents. Most recent pharmacokinetic studies of DNA vaccines have employed QRT-PCR (11–13), which regulatory guidelines describe as the most common and reliable means for assessing plasmid levels in biodistribution studies among the available nucleic acid-detection techniques (14,15).

The empirically determined detection limit of our QRT-PCR assay was 10 copies/200 ng gDNA in all tissues tested, a limit that satisfies regulatory agency guidelines. In the USA, Food and Drug Administration (FDA) guidelines stipulate a QRT-PCR detection limit for DNA vaccine of <100 copies/μg of host DNA (14). With a detection limit of 50 copies/μg gDNA (Fig. 2) and CV values less than 4.0% (Table I), our QRT-PCR assays performed with high sensitivity and reproducibility.

In the pharmacokinetic study, the HPV16L1 gene intravenously injected in the AcHERV form showed longer blood circulation compared with the naked plasmid DNA vaccine. The pharmacokinetic profile of naked plasmid DNA described here is consistent with previous reports, which have shown that naked plasmid DNA is rapidly degraded in the blood (within 24 h)

after administration via the intravenous route (16). The 272.2-fold higher MRT of HPV16L1 given intravenously in AcHERV might be explained, in part, by protection afforded by the AcHERV vector against nuclease-mediated degradation in the blood. The relatively higher stability of AcHERV-HPV16L1 in the blood may contribute to the persistence of HPV16L1 DNA levels in the blood after intramuscular administration. HPV16L1 gene intravenously injected in the AcHERV form showed notably different pharmacokinetic profiles from previously reported plasmid DNA vaccines (17,18). As compared to pseudorabies virus glycoprotein gB-encoding plasmid DNA vaccines (17), AcHERV-based HPV16L1 DNA vaccines showed 321-fold higher MRT values, and 7.7-fold higher bioavailability. Moreover, AcHERV-based HPV16L1 DNA vaccines provided bioavailability more than 3 orders of magnitude higher in comparison with human immunodeficiency virus-specific DNA vaccines intramuscularly administered in mice (18).

Compared to naked HPV16L1 plasmids, AcHERV-based HPV16L1 DNA vaccines exhibited lower clearance rates in muscle tissues (Fig. 3). DNA vaccines delivered in nonviral nanocarriers, such as cationic liposomes (19) or polymers (16,20), have been reported to persist longer at the injection site. These nanocarriers might protect plasmid DNA from serum- and tissue-specific nucleases. The persistence of other naked plasmid DNA vaccines in muscle tissues has been shown to vary depending on the DNA vaccine dose and type. Following intramuscular administration of different doses of plasmid DNA encoding the *Mycobacterium leprae* 65-kDa heat shock protein, plasmid DNA at a dose of 100 μg/mouse was detected in muscle for up to 180 days but a dose of 20 μg/mouse was undetectable in muscle after 30 days (21). Following intramuscular administration of 10 μg (10^{12}–10^{13} copies) of the plasmid pDNAX (pVAX-Hsp60 TM814) vaccine encoding the heat shock protein 60 in mice, 1 year was required for the levels of naked plasmid DNA in muscle tissues to decrease to below quantification limits (19). In the case of pSO2C1 DNA vaccine harboring the *Bacillus thuringiensis cry11Bb* gene, it was reported that plasmid DNA persisted in the muscle of mice for up to 2 years after intramuscular administration of a dose of 5 μg (22).

Additional studies will be required to determine whether the retention of HPV16L1 gene in muscle tissue is due to the integration of genes into chromosomes. FDA guidelines require that integration studies be performed when the copy numbers of DNA vaccine are greater than 30,000 per microgram of host DNA 90 days after administration (14). Although our HERV-based DNA vaccines persisted longer than naked plasmid vaccine at the injection site, the levels of HPV16L1 gene were already less than 1,100 copies per microgram gDNA 30 days after intramuscular administration. These results indicate that there would be little likelihood that the HPV16L1 gene integrated into host chromosomes.

AcHERV-based DNA and naked plasmid DNA vaccines showed different biodistribution patterns after intramuscular injection. Overall, AcHERV-based DNA vaccines showed higher retention in all tissues tested, but showed no tissue-specific tropism. Tissue-specific biodistribution patterns have been observed with previously tested viral vector-based DNA vaccines. For example, adenovirus type 5-vectored DNA vaccine was found to be biodistributed only to spleen and liver through binding to coxsackievirus and adenovirus receptor (23). Recombinant vesicular stomatitis virus expressing HIV-1 Gag showed greater persistence in lymph nodes compared to other tissues after intramuscular administrations (24).

Notably, examination at various times after administration via the intramuscular route revealed systemic tissue distribution and prolonged retention of the AcHERV-based DNA vaccine (Fig. 4b). HPV16L1 in AcHERV may distribute to organs other than muscle via the lymphatic network after intramuscular injection. The Räty group reported that baculovirus may spread via the lymphatic network to other organs after administration via different routes (i.e., intramuscular, intraperitoneal, and intracerebroventricular) in rats (25).

Because the clearance rates of the HPV16L1 gene were generally slower after delivery in AcHERV compared to that of the naked form, a long-term biodistribution study is currently underway to confirm the time required for the total clearance of HPV16L1 gene after administration in AcHERV.

CONCLUSION

We demonstrated that the pharmacokinetic and biodistribution profiles of the AcHERV-based HPV16L1 vaccine differ significantly from those of naked plasmids. This study may provide a framework for understanding the pharmacodynamics and safety of AcHERV-based vaccines.

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