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Research Paper

In Vivo Human MCP-1 Transfection in Porcine Arteries by Intravascular Electroporation

Randolph W. Seidler,[1] Susanne Allgäuer,[2] Susanne Ailinger,[2] Andreas Sterner,[2] Nagendu Dev,[3] Dietmar Rabussay,[3] Henri Doods,[2] and Martin C. Lenter[2,4]

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Purpose. The purpose of this study was to develop a nonviral gene transfer method for therapeutic delivery of the human monocyte chemoattractant protein-1 (MCP-1) in patients with peripheral artery disease, using local catheter-mediated electrotransfer of naked plasmid DNA into arteries.
Methods. Arterial walls of the *A. profunda femoris* of pigs were transfected either with a human MCP-1 or with a firefly luciferase-encoding DNA construct. The efficacy of electrotransfer of DNA was analyzed after 2 days by quantitative polymerase chain reaction (PCR) or luciferase activity measurements. To optimize MCP-1 gene transfer conditions, a voltage range of 60–150 V was applied as a train of six square pulses of 20 ms each at 1 Hz and was combined with a dose of 150 μg DNA. Subsequently, the optimized voltage was used to test a dose range of 80–300 μg DNA.
Results. The voltage optimum for arterial transfection was observed at 80 volts. Using this setting, the dose application of 300 μg MCP-1 plasmid DNA (the maximal dose tested) demonstrated the highest MCP-1 expression signal. The electric pulses and the transfer and expression of human MCP-1 per se did not induce endogenous porcine MCP-1 expression in treated arteries. Interestingly, angioplastic predilation of the artery before gene transfer, which had originally been postulated to enhance transfection by improving access of the plasmid to subendothelial cell layers, resulted in an attenuated transfection efficacy.
Conclusions. The present study demonstrates that transluminal catheter-based electroporation provides an efficient technology for nonviral intravascular gene transfer by just applying unformulated DNA.

KEY WORDS: arteriogenesis; electroporation catheter; MCP-1; naked DNA; pigs; vascular gene therapy.

INTRODUCTION

Peripheral arterial disease (PAD) is a progressive occlusive disease of peripheral arteries predominantly in the leg. It has been estimated that approximately 20–30% of the elderly population are affected by PAD (1–4). Pharmacologic therapy of PAD is usually limited to risk-factor modification (i.e., cholesterol or blood pressure lowering for example by statins or angiotensin-converting enzyme inhibitors, respectively), vasodilatation using prostaglandin analogs, and prevention of thrombotic complications (5,6). Because surgical revascularization or percutaneous transluminal angioplasty (PTA) is not possible in all patients and results in a higher restenosis rate compared to the coronary circulation, an effective nonsurgical therapy may provide improved revascularization and preservation of limbs. Therefore, a "biological" revascularization by means of angiogenesis or arteriogenesis appears to be an attractive long-term alternative. Monocyte chemoattractant protein-1 (MCP-1) is a potent arteriogenic stimulant in rabbit and pig femoral artery ligation models leading to improved peripheral blood flow by stimulating development of collateral arteries (7–9). In peripheral ligation models, MCP-1 protein was mainly applied via continuous intra-arterial infusion over days up to weeks (10–15). Because these studies indicate that MCP-1 plays a central role in the process of arteriogenesis, local infusion of MCP-1 protein has been proposed as a potential treatment for peripheral artery disease, coronary artery disease, and cerebral occlusive disease. In a clinical setting, administration of MCP-1 via long-term intra-arterial application appears undesirable. Long infusion times are expected to be not well tolerated by the patients because of the risk for damaging the vessel by the infusion catheter (potentially resulting in further stenosis), the risk of thrombus formation, and for reasons of patient compliance. Therefore, overexpression of MCP-1 after local

[1] Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, Connecticut 06877, USA.
[2] Boehringer Ingelheim Pharma GmbH & Co. KG, Birkendorfer Str. 65, 88397, Biberach, Germany.
[3] Genetronics Inc., San Diego, California 92121, USA.
[4] To whom correspondence should be addressed. (e-mail: martin.lenter@boehringer-ingelheim.com)

ABBREVIATIONS: EP catheter, electroporation catheter; FAM, 6-carboxy-fluorescein; GAPDH, glycerolaldehyde-3-phosphate dehydrogenase; JOE, 6-carboxy-4′,5′-dichloro-2′,7′-dimethoxy-fluorescein; ms, milliseconds; RLU, relative light units; TAMRA, 6-carboxytetramethylrhodamine.

gene delivery may be of substantial benefit to patients suffering from vascular occlusive diseases.

Intravascular delivery of the MCP-1 gene can be carried out within minutes using a catheter device that is inserted into the blood vessel of the patient and advanced to a preselected location of blood vessel, which has been selected for gene transfer. Various catheters useful for local intravascular delivery of DNA are known, including hydrogel balloon, laser-perforated balloon ("Wolinsky" balloon), weeping, channel and Dispatch balloons, and variations thereof, but these are mostly used to deliver formulated DNA (16–20).

Because of the potential limitations for viral and lipoplex/polyplex-based nonviral approaches outlined above, we developed an electric field-mediated intra-arterial gene transfer method for unformulated plasmid DNA in large animals by using an electroporation catheter (Genetronics, Inc., San Diego, CA, USA). This device consists of a microporous balloon serving as the DNA reservoir and an electric field generated by electrodes for vascular DNA transfer (21,22).

MATERIALS AND METHODS

Vectors

The pcDNA3 backbone (Invitrogen, Groningen, Netherlands) was used to construct pAH7 plasmid (control plasmid) after digestion with *Bbs*I and *Bsm*I to excise the neo-cassette; the *Bsm*I site was destroyed and the *Bbs*I site restored. The pcDNA3 ampicillin-resistance cassette was replaced by the kanamycin-resistance cassette, amplified from pZErO-2 (Invitrogen). The 953-bp PCR fragment was inserted in the *Bsp*HI site to create pAH7. The genomic MCP-1 sequence (accession no. M37719.1) was amplified from chromosomal HeLa cell DNA to construct pRZ-hMCP-1-genomic (MCP-1_genomic) using the following primers:

hMCP-1-F1: 5′-NNN-NNN-AAGCTTCACTCTCG CCTCCAGCATGAA-A-3′

hMCP-1-R1: 5′-NNN-NNN-GATATCGGGTTGTG GAGTGAGTGTTCA-A-3′

Identity of the 1.5-kb MCP-1 PCR fragment was verified by sequencing. The PCR fragment was digested with *Eco*RV and *Hin*dIII and cloned into pAH7. The resulting vector pRZ-hMCP-1-genomic expresses the MCP-1 gene under control of the cytomegaly virus (CMV) promoter.

The luciferase gene (firefly) was cloned via *Eco*RV and *Hin*dIII restriction sites into pAH7 to yield a reporter gene plasmid. The resulting vector pAH7/Luc contained the same vector backbone as pRZ-hMCP-1-genomic but expressed firefly luciferase instead of MCP-1 under control of the CMV promoter.

Animal Model

The study protocol was approved by the local animal care and use review committee. It conformed to the *Guide for the Care and Use of Laboratory Animals* (NIH publication No. 85-23, revised 1996). Experiments were performed in Goettingen minipigs (20–25 kg; Ellegaard, Ringsted, Denmark). Pigs were anesthetized using intramuscular injections of ketamine in combination with either azetylpromazine or midazolam. The animals were intubated and anesthesia was maintained using isoflurane. After sterile surgical preparation, the carotid artery was surgically exposed, a catheter-introducer sheath was inserted, and an angiographic catheter (8F) was advanced to the aortic bifurcation. Under fluoroscopic control, the catheter was further advanced into the proximal part of the *A. profunda femoris*, and an arteriogram was performed using radiocontrast dye. The diameter of the lumen of the *A. profunda femoris* was measured, and an electroporation catheter slightly larger in diameter was chosen for the transfection. The electroporation balloon catheter (Genetronics Inc.) consisted of a shaft and a microporous balloon of 20-mm length and balloon diameters between 3.5 and 4.5 mm. The micropores allow for the egression of the plasmid. The balloon was connected to an injection port at the proximal end through a channel inside the shaft. At the tip, there were two negatively charged electrodes slightly outside the balloon on either side and a positively charged electrode inside the balloon to generate the electric field required for electroporation. The electrodes were connected to an external voltage source delivering the pulses. The electroporation catheter filled with phosphate-buffered saline (PBS) containing the expression plasmid solution (pAH7/Luc for the expression of luciferase or MCP-1-genomic for the expression of MCP-1 protein) was advanced into the proximal part of the *A. profunda femoris*. The vector solution was injected, and the electroporation was performed simultaneously or shortly after the injection of the respective plasmid with the balloon still being inflated. The electroporation protocol consisted of six square pulses of 20-ms duration each delivered at a frequency of 1 Hz and at a voltage of 60 V for pAH7/Luc and (in different experiments) ranging from 60 to 150 V for MCP-1-genomic. The expression vector dose was 100 μg for pAH7/Luc and varied from 80 to 300 μg for MCP-1-genomic. The catheter was then removed, the surgical wound was closed, and the animals were allowed to recover.

Two days later, the animals were reanesthetized using the same protocol as above, the bifurcation of the iliac artery into the femoral artery and the *A. profunda femoris* was exposed, the animals were heparinized, and the proximal 3 cm of the profundal artery was dissected. The two-day time point was chosen because in prior experiments and own pilot experiments (data not shown) using other gene transfection technologies, strong luciferase signals and target mRNA signals have been obtained at this point in time (23). For the luciferase measurements, the arteries were put into PBS, snap frozen in liquid nitrogen, and stored at −80°C until the analysis. The arterial samples for the quantitative mRNA analysis were immediately immersed in RNA*later* (Qiagen, Hilden, Germany) for the conservation of the RNA, fixed for at least 1 day at 4°C, and then frozen until the analysis.

For angioplastic pretreatment of the transfection site, the left *A. profunda femoris* was first dilated (5 atm for 60 s) using an angioplasty catheter (Maverick™, Boston Scientific Scimed, Inc., Maple Grove, MN, USA) using the same size of catheter as the electroporation catheter (diameter 3.5–4.5 mm, length 20 mm). After the dilatation, the angioplasty catheter was removed and the electroporation catheter was

advanced over the guidewire to the same site followed by transfection using pAH7-Luc (100 μg DNA, 60 V, six square pulses, each of 20 ms, 1 Hz). The catheter was then removed, the guidewire repositioned in the contralateral leg, and the right *A. profunda femoris* was transfected using a new electroporation catheter, the same plasmid dose, and EP settings. Two days after the transfection, the animals were reanesthetized using the same protocol and the arterial segments were removed.

Luciferase Assay

To measure luciferase activity, artery specimens were thawed prior to homogenization and 1 ml homogenization buffer (250 mM Tris/HCl, pH 7.5, with 0.9% saline) was added per 10 mg wet weight of tissue. The tissue was cut into smaller pieces before being homogenized stepwise in the test tube using an Ultra Turrax homogenizer (ICA, Staufen, Germany). Subsequently, the homogenate was centrifuged for 10 min at 4°C, and the supernatant was collected. For measurement, 100 μl homogenate or homogenate dilutions (diluted with homogenization buffer) were added to 150 μl luciferase assay buffer (25 mM Tricine, 0.5 mM EDTA, 0.54 mM NaTPP, 16.3 mM $MgSO_4$, 1.2 mM ATP, 0.05 mM luciferin, 56.8 mM 2-mercaptoethanol, and 0.1% Triton X-100), and chemiluminescence was measured by a Top Count Reader (Packard BioScience Company, Groningen, Netherlands) in intervals of 1 s. Recorded relative light units (RLU) were calibrated to 1 mg of protein.

Quantitative PCR Analysis

For TaqMan analysis, the preparation of total RNA from pig arteries was performed using the RNeasy Protect Mini Kit from Qiagen following the protocol for isolation of total RNA from heart, muscle, and skin tissue. After dissection, arteries were cleaned from blood, connective tissue was removed, and specimens were placed in a 2-ml reaction tube containing at least 1.8 ml RNA*later* and stored at 4°C either overnight or for a few days until further processing. For extended storage exceeding a few days, the samples were transferred to −20°C. For homogenization, the

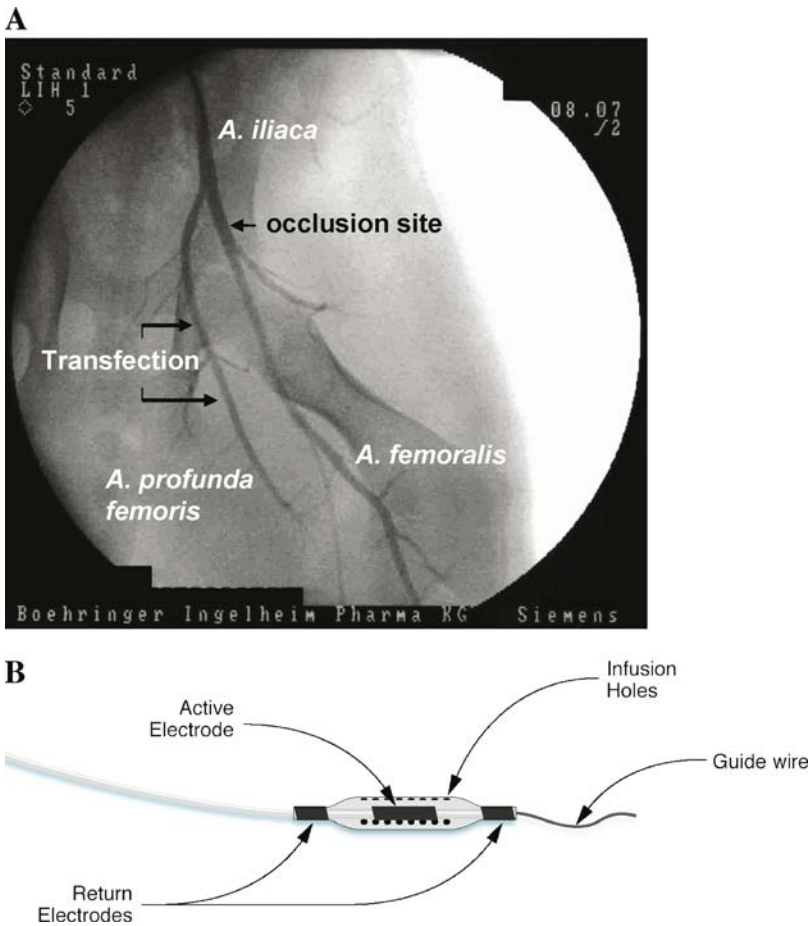

Fig. 1. Transfection of porcine arteries by intravascular electroporation. (A) Arteriogram of the left hind limb of an anesthetized minipig indicating the potential transfection site in the *A. profunda femoris*. (B) Schematic picture of the electroporation catheter device (Genetronics Inc.) depicting the microporous balloon with the three electrodes between which the electrical field is generated—one inside (active electrode) and one on either side outside the balloon (return electrodes).

tissues were minced and transferred into a BioPulverizer (Qbiogene, Heidelberg, Germany), and subsequent RNA preparation was performed according the Qiagen protocol.

For MCP-1 expression analysis, the TaqMan EZ RT-PCR Core reagents (Applied Biosystems, Germany) were used. Specific primers for human MCP-1 (forward primer: ATA GCA GCC ACC TTC ATT CC; reverse primer: TGC ACT GAG ATC TTC CTA TTG G and 3′ FAM/5′ TAMRA double-labeled oligonucleotide probe TCG CTC AGC CAG ATG CAA TCA ATG), for porcine MCP-1 (forward primer: TAC CAG TAA GAA GAT CTC GAT; reverse primer: AGA TCA CTG CTT CTT TAG GAC ACT T and 3′ FAM/5′ TAMRA double-labeled oligonucleotide probe: CTG ATG AGC TAC AGA AGA GTC ACC AGC AGC), as well as primers for porcine glyceraldehyde-3-phosphate dehydrogenase (GAPDH) control (forward primer: GCA CAG TCA AGG CTG AGA ATG; reverse primer: CTT CTC CAT GGT CGT GAA GAC and 3′ JOE/5′ TAMRA labeled probe CAG GAG CGA GAT CCC GCC AA) were purchased from Eurogentec (Seraing, Belgium). Samples were analyzed in triplicates using 100 ng total RNA for each reaction.

Reverse transcription of the RNA and subsequent real-time PCR was performed in an ABI Prism 7700 Sequence Detection System using a two-step PCR reaction consisting of an initial denaturation step (94°C for 5 min) and 40 repetitions of a denaturation (94°C for 20 s) and a combined annealing and elongation step (59°C for 1 min). Results were expressed as mean values. A calibration curve obtained by dose range of total RNA from a human MCP-1 control transfection (standard) was carried out for each experiment and used to determine the specific GAPDH and MCP-1 signals. The specific signal derived from the MCP-1 mRNA is expressed in percent and refers to the signal derived from GAPDH mRNA in the same sample that is set to 100%.

Statistical Analysis

Result are expressed as mean ± SEM. Statistical analysis was performed using a one-way ANOVA combined with Newman–Keuls posttest using Prism 4 (GraphPad Software, Inc., San Diego, CA, USA).

RESULTS

Human MCP-1 Gene Delivery Into Porcine Arteries by an Electroporation Catheter

In a new approach for intravascular gene transfer, we tested an electroporation catheter for the transfection of the *A. profunda femoris* in pigs (Fig. 1A and B). To evaluate the optimal voltage for electrotransfer, a constant amount of 150 μg DNA noncomplexed pRZ-hMCP-1-genomic vector (MCP-1-genomic), containing the human MCP-1 gene, was transfected using different voltages ranging from 60 to 150 V. Transfer efficacy to porcine arteries was determined 2 days later by the quantitative PCR analysis of expressed human MCP-1 mRNA as depicted in Fig. 2A ($n = 4$ per group Ctrl, 60–100 V; $n = 2$ per group, 150 V). A detectable PCR signal for the human MCP-1 transcript was found in every transfected *A. profunda femoris*. The optimum voltage for electrotransfection was reached at 80 V, and the relative human MCP-1 signal was lower after administration of higher voltages.

The influence of the DNA dose on gene transfer efficacy to porcine arteries was tested by applying a dose range from 80 to 300 μg MCP-1-genomic at a constant voltage of 80 V. Quantitative PCR analysis ($n = 4$ per group) revealed a transfection maximum at the highest dose of 300 μg MCP-1-genomic but no clear dose dependency (Fig. 2B). These results suggest 80 V and a minimum of 300 μg naked plasmid DNA as

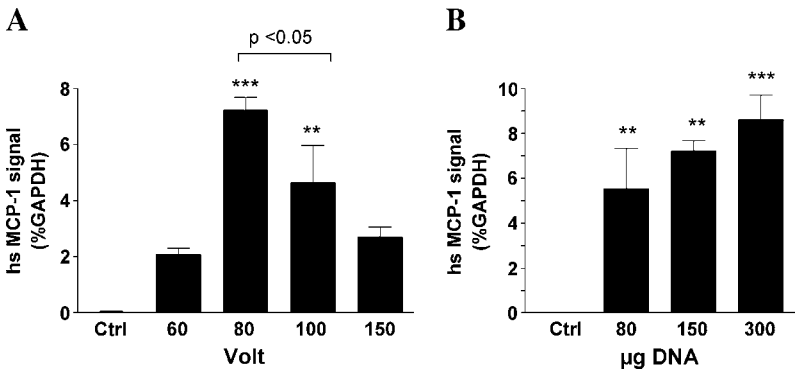

Fig. 2. Quantitative polymerase chain reaction (PCR) analysis of human monocyte chemoattractant protein-1 (MCP-1)-genomic expression in electrotransfected pig arteries. (A) Transfection of porcine femoral profunda arteries with 150 μg naked human MCP-1-genomic plasmid by the electroporation catheter at voltages ranging from 60 to 150 V. Expression of human MCP-1 mRNA was monitored 2 days later by quantitative PCR and is relatively expressed in percent each referring to an internal glyceraldehyde-3-phosphate dehydrogenase (GAPDH) reference signal that was set to 100%. Untreated porcine arteries served as controls (Ctrl). Mean ± SEM, $n = 4$; 150 V, $n = 2$; $^{***}p < 0.001$ and $^{**}p < 0.01$ referred to control. (B) Transfection of porcine femoral profunda arteries with 80, 150, and 300 μg uncomplexed human MCP-1-genomic plasmid by the electroporation catheter at constant voltage setting of 80 V. Expression of human MCP-1 mRNA was monitored 2 days later by quantitative PCR and is relatively expressed in percent each referring to an internal GAPDH reference signal that was set to 100%. Untreated porcine arteries served as controls (Ctrl). Mean ± SEM, $n = 4$; $^{***}p < 0.001$ and $^{**}p > 0.01$ referred to control.

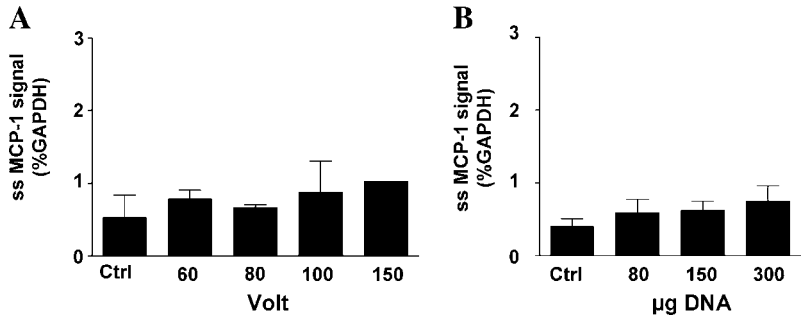

Fig. 3. Quantitative PCR analysis of endogenous porcine MCP-1 expression in electrotransfected and untransfected pig arteries. (A) Electroporation of porcine femoral profunda arteries with 150 µg naked human MCP-1-genomic plasmid at voltages ranging from 60 to 150 V. Expression of porcine MCP-1 mRNA was monitored 2 days later by quantitative PCR and is relatively expressed in percent, each value referring to the internal 100% GAPDH signal. Untreated porcine arteries served as controls (Ctrl). Mean ± SEM, $n = 3$; 150 V, $n = 1$. (B) Electroporation of porcine femoral profunda arteries with 80, 150, and 300 µg naked human MCP-1-genomic plasmid at 80 V. Expression of porcine MCP-1 mRNA was monitored 2 days later by quantitative PCR and is relatively expressed in percent, each value referring to the internal 100% GAPDH signal. Untreated porcine arteries served as controls (Ctrl). Mean ± SEM, $n = 4$.

the optimal setting for intravascular gene transfer to porcine arteries using the Genetronics electroporation catheter device.

Endogenous Porcine MCP-1 Expression Following Gene Transfer

To address potential endogenous MCP-1 expression in porcine arteries, either constitutively induced by the electroporation stimulus or potentially stimulated by human MCP-1 expressed in the same vessel, mRNA extracted from transfected arteries was subjected to a quantitative PCR analysis for porcine MCP-1. However, no differences in endogenous porcine MCP-1 expression after electroporation compared to untreated vessels were found, and porcine MCP-1 expression remained at or below 1% of GAPDH control. This was irrespective of electrotransfer using a constant amount of MCP-1-genomic DNA at different voltages, ranging from 60 to 150 V (Fig. 3A), or of various amounts of MCP-1-genomic vector DNA, ranging from 80 to 300 µg, at a constant voltage of 80 V (Fig. 3B). These results indicate that neither electroporation nor the application and expression of transferred MCP-1-genomic vector did lead to an increase of porcine MCP-1 expression.

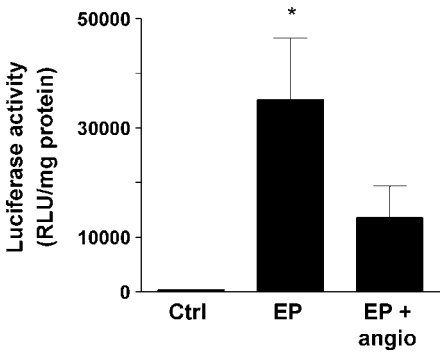

Fig. 4. Luciferase signal (relative light units normalized to mg protein) in the arteries in which angioplasty was performed prior to the catheter-mediated electroporation (EP + angio) and in the arteries that were only electroporated without pretreatment (EP). One of each artery was harvested from each animal. Untreated arteries served as control (Ctrl). Mean ± SEM, $n = 4$; $*p > 0.05$ referred to control.

Electrotransfection of Arteries Following Angioplastic Pretreatment

To study, if predilation of the *A. profunda femoris* at the transfection site before electroporation results in an altered transfection efficacy, we predilated the left *A. profunda femoris* using an angioplasty catheter (Maverick, Boston Scientific) followed by electrotransfer of 100 µg luciferase reporter plasmid (pAH7/Luc) delivered at 60 V using the Genetronics electroporation catheter. Using a new electroporation catheter, the same amount of reporter DNA was transferred at the same setting to the *A. profunda femoris* of the contralateral leg without prior angioplasty. Determination of luciferase activity in the transfected artery specimen after 2 days showed that angioplastic treatment before electrotransfection markedly reduced the reporter gene expression, putatively by reducing the compression on the balloon leading to a decreased plasmid uptake. However, because of the small number of experiments, the difference between the two groups with and without angioplasty pretreatment failed to show statistical significance (Fig. 4). This suggests that angioplastic pretreatment of porcine arteries potentially reduced gene transfer efficacy of the electroporation catheter.

DISCUSSION

This study investigated the use of intravascular gene transfer in pigs by electroporation using a human MCP-1 or a luciferase reporter gene construct. We found optimized settings for voltage and gene dose with a low variability in gene expression between individual experiments. Using this catheter, electrotransfer of DNA to porcine profunda femoral arteries resulted in all cases in the expression of the

transferred target genes as assessed by quantitative PCR or luciferase activity, irrespective if a human MCP-1 or a firefly luciferase encoding DNA construct was used. The transfection optimum was observed at 80 V using 300 μg plasmid DNA as the maximum dose applied. Expression of endogenous porcine MCP-1 was not induced, neither by electroporation nor following transfer and expression of human MCP-1. Because of the potential limitations inherent in viral and nonviral gene transfer approaches using complexed DNA, development of transluminal gene transfer by catheter-based electroporation could provide a major advantage for gene therapeutic treatment of vascular diseases. Monocyte chemoattractant protein-1 has been shown to induce collateral artery growth in rabbits through a monocyte-dependent process (8). Our group has recently shown that MCP-1 exerts similar effects in pigs, a species that is closer to humans in size and arterial dimensions than small laboratory animals (15). However, the use of a prolonged intra-arterial infusion is limited by the risk for thromboembolic complications and patient compliance. Therefore, gene therapy with the aim of local MCP-1 production by cells of the blood vessel, secreting the protein over days into the blood stream and thus mimicking the intra-arterial infusion, may be a viable alternative. Through this approach, a short surgical intervention would suffice, potentially reducing the risk for complication and increasing compliance of the patient. We have shown that gene transfer of the MCP-1 plasmid using a lipoplex vector system induced an increase in arterial conductance comparable to that observed after protein infusion (24). However, to reduce the complexity of the gene transfer system to the delivery of uncomplexed DNA while maintaining high and consistent expression levels, we investigated alternative methodologies.

The choice of a suitable *in vivo* gene transfer system, however, is a critical step in the development of any gene therapeutic approach. Very often, it is necessary to make a choice between efficiency and safety: viral transfection vectors such as adenovirus, adeno-associated virus, or retrovirus derived vectors may, under certain transfection conditions, provide high transfection rates, but the potential safety concerns connected with these approaches are far from being resolved (25,26). On the other hand, gene transfer using lipoplexes, polyplexes, and similar nonviral transfection agents is relatively safe, but transfection rates are often unsatisfactory (27). Electroporation is a technology, which utilizes short electrical, usually rectangular, pulses generating an electric field to facilitate the uptake of DNA constructs into cells. The porous catheter, which was used in this study, is virtually identical in design to other balloon catheters used in patients with the exception that it is equipped with electrodes to generate the electrical field. To our knowledge, this is the first study systematically investigating the use of an intra-arterial electroporation catheter for gene transfection. Two previously conducted studies tested the feasibility of this technique and showed that the gene expression after transfection is found in all layers of the vascular wall (22,28). Another study investigated electroporation *in vivo* using caliper electrodes, which were applied externally to an artery, a method which is of doubtful value for use in patients (29).

In this study, we investigated the effect of different voltages ranging from 60 to 150 V on gene transfer efficacy to porcine arteries that share in terms of size and wall thickness many similarities with that of humans. This range was chosen based on theoretical calculations of the field strengths generated by the electroporation catheter and confirmed in pilot experiments (22). We found a bell-shaped voltage–transfection relationship, which confirms previous studies using external electroporation. The underlying cause for the nonlinear relationship is not known at present but may be related to a decreased tolerability of cells to higher voltages. We found that with 80 V, a maximal transfection efficiency was achieved. This was significantly higher than the previously reported 20 V in rabbit arteries using caliper electrodes (29). However, one has to keep in mind that because of the differences in the method by which the electric field was applied (catheter *vs.* caliper electrodes), the field strength within the vascular tissue could be significantly different. Also, because of the difference in arterial wall thickness and vessel size in pigs *vs.* rabbits, a different voltage setting may be necessary for optimal transfection, possibly highlighting the potential pitfalls extrapolating from rabbits to man. Based on this result, 80 V was used in the subsequent experiments.

The next step was to investigate the dose–expression relationship of the applied plasmid. Again, we found a reproducible and significant expression of the hMCP-1 mRNA, which was similar in magnitude to that observed in the first study. Notably, we did observe similar expression between 80 and 300 μg DNA, indicating that above 80 μg, the amount of DNA is not limiting and may be already at the plateau of the dose–response relationship. At the same time, endogenous (porcine) MCP-1 was not induced by electroporation, irrespective of the voltage or DNA dose applied, suggesting that the possible stress of the procedure did not induce endogenous porcine MCP-1 expression in the treated arteries, whereas others have found up-regulation of MCP-1 mRNA after severe balloon injury in rats (30). This finding suggests that the trauma or denudation induced using this procedure is comparatively low.

Frequently, catheter-based interventions involve angioplasty, and it is perceivable that future intravascular gene transfer will be performed in conjunction with a balloon dilatation in some patients. We therefore investigated the effect of angioplasty on the transfection efficiency when performed prior to the gene transfer. We found that the transfection efficiency was reduced, although one could speculate that prior dilatation of the artery may enhance the access of the plasmid to the interstitial space. The reason for this may potentially be based on the fact that the balloon diameter used for the angioplasty had the same diameter as that of the electroporation balloon. One could speculate that following predilatation of the vessel, the contact of the vascular wall to the porous membrane of the EP catheter is not as tight as in undilated arteries hampering the transfer of DNA to the vascular wall. Future studies aiming at the use of larger EP catheters following angioplasty may clarify this issue.

In the present study, the parameters that were expected to have the highest influence on the transfection efficiency (gene dose and voltage) were investigated. Application of these optimized settings led to a robust and reproducible gene expression. However, it cannot be excluded that some of the other parameters such as pulse duration, pulse frequency,

monophasic *vs.* biphasic pulse shape, or the absolute number of pulses may have an additional influence on the transfection efficiency. Pilot experiments in rabbits using the catheter-based delivery did not indicate that these parameters had a significant influence on gene expression, but subtle differences cannot be excluded. Others have found only modest differences between 5- and 20-ms pulse length; thus, the applied 6 ms was well within this range (29). However, further investigations may be warranted.

In conclusion, the present study demonstrated the efficiency of *in vivo* electroporation as a means for gene transfer to the vascular wall in pigs and showed that this can be facilitated by a transcutaneous intraluminal approach. These findings suggest that this method may be a clinically feasible procedure, which is able to reliably and reproducibly transfect arteries with a low intraindividual variability.

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