Increase in ambient temperature and laser enhance gene transfer

Hongbao Ma[1,*], George Chen[2], Jenny Young[2], Shen Cherng[3]

[1]*Department of Bioengineering*, *Zhengzhou University*, *Zhengzhou*, *Henan* 450001, *China*; [2]*Michigan State University*, *East Lansing*, *MI* 48824, *USA*; [3]*Department of Electrical Engineering*, *Chengshiu University*, *Niaosong*, *Taiwan* 833, *China*

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Abstract

Several techniques are currently used to transfer genes into various cells, tissues and organs. Although gene therapy is a potential therapeutic approach for arterial restenosis and angiogenesis, the efficiency of transfection is relatively low. Human aorta smooth muscle cells were cultured in F12K medium. Swine growth hormone gene transfection was performed by calcium phosphate coprecipitation at various temperatures: 23 ºC, 37 ºC and 43 ºC. Swine growth hormone expression was detected using an indirect ELISA. The heated cultured human aorta smooth muscle cells had a significantly higher expression of the transfected growth hormone gene. Ambient temperature rise to 43 ºC for 30 minutes provided greater transient transfection of the swine growth hormone gene when compared to ambient temperatures at 37 ºC and 23 ºC ($P < 0.01$). The greatest effects occurred within 10 minutes of incubation and persisted up to 30 minutes. The results suggest that even a few degrees of ambient temperature rise can significantly increase gene transfer into smooth muscle cells. This may be of value when using gene therapy with transfection procedures. [Life Science Journal. 2008; 5(1): 46 – 50] (ISSN: 1097 – 8135).

Keywords: cell; gene transfer; hormone; temperature

1 Introduction

Gene therapy is the deliberate transfer of DNA for therapeutic purposes. Transplantation procedures involving bone marrow, kidney and liver are not considered a form of gene therapy. The concept of transfer of genetic information as a practical clinical tool arose from gene cloning technology developed during 1970s (Bechtel, 1979), however, gene therapy has reached a crossroads during the past years (Matsui, 2003). Gene transfer is one of the key factors in gene therapy and the main challenge is to perform gene transfer efficiently and safely (Dick, 1992; Young, 2002; Goya, 2004). Abortive attempts were made in the early 1980s to treat two patients with thalassaemia and these experiments were surrounded by controversy as the pre-clinical evidence of effectiveness was not adequate and full ethical approval had not been given (Temple, 1982). Various methods including use of a viral vehicle, attachment to lipofection (Young, 2002), calcium phosphate coprecipitation (Sambrook, 1989; Ausubel, 1992), electroporation, and DEAE-dextran transfection (Puchalski, 1992), gene gun (biolistics), laser, temperature enhancing gene transfer (Ma, 2004a; Ma, 2004b), and the other methods have been reported with limited success (Temple, 1982; Goya, 2004). Without the ability to isolate and replicate defined genetic sequences it would be impossible to produce purified material for clinical use. There is a further implication in that it involves only specific sequences containing relevant genetic information. The drive for the practical application of this technology comes from the biotechnology industry, with its quest for complex human biomolecules produced by recombinant techniques in bacterial. Within a decade, pharmaceutical-grade insulin, interferon, interleukin-2 (IL-2) and tumour necrosis factor (TNF) were all undergoing clinical trials. The next step is to obtain gene expression in vivo. Genetic disorders are the obvious first

*Corresponding author. Tel: 01186-156-1769-1256; Email: hongbao@gmail.com,

target for such therapies.

The electrical conductance change in the nasal epithelium after insertion of the cystic fibrosis trans-membrane regulator gene is a good example. Gene electrotransfer is an efficient and reproducible nonviral gene transfer technique useful for the nonpermanent expression of therapeutic transgenes. Ferreira et al established optimal conditions for the electrotransfer of reporter genes into mesenchymal stem cells isolated from rat bone marrow by their selective adherence to tissue-culture plastic ware. In their study, the electrotransfer of the lacZ reporter gene was optimized by adjusting the pulse electric field intensity, electric pulse type, electropulsation buffer conductivity and electroporation temperature. LacZ electrotransfection into MSCs was optimal at 1500 V/cm with pre-incubation in Spinner's minimum essential medium buffer at 22 ºC (Ferreira *et al*, 2008).

Laser-mediated gene transfection has received much attention as a new method for targeted gene therapy because of the high spatial controllability of laser energy. It was reported that both *in vivo* and *in vitro* that plasmid DNA can be transfected by applying nanosecond pulsed laser-induced stress waves, and it was found that change in ambient temperature in a specific range resulted in drastic change in transfection efficiency for NIH 3T3 cells, and cellular heating increased transfection efficiency for nonmalignant cells, while heating decreased transfection efficiency for malignant cells (Terakawa *et al*, 2006).

For the features of a suitable target disease for gene therapy approaches, certain factors should be considered. The disease must be life threatening so that the potential risk of serous side effects is ethically acceptable. The gene must be available and its delivery to the relevant tissue feasible. This may involve the ex vivo transfection or transduction of cells removed from a patient, which are returned after maniputation. This approach is only possible with a limited range of tissues and most trials so far have used bone marrow. Ideally, a short-tern surrogate end-point to demonstrate the physiological benefit of the newly inserted gene should be available. The electrical conductance change in the nasal epithelium after insertion of the cystic fibrosis trans-membrane regulator gene is a good example. There must be some possibility that the disability caused by a disease is reversible. Some of the tragic mental and physical handicaps caused by some genetic metabolic disorders may never be improved by somatic gene therapy, however successful a gene transfer protocol (Sapolsky, 2003). If the necessary requirement of the gene therapy happens, the effective of the gene transfection will be the limitation factor for the practice. Unfortunately, there is not matured technique for the efficiency and safety gene transfer. For example, the leukaemia cases generated by gene transfer using viral vehicle technique was reported (Cavazzana-Calvo, 2004).

This project is to determine if ambient temperature will enhance gene transfection into human smooth muscle cells in culture, and it can be a reference for the gene therapy. Even with ethnic and safety concern, nobody can prevent the development of the animal clone science (Ma, 2004c).

2 Materials and Methods

2.1 Cell culture

Human aorta smooth muscle cells were cultured in F12K medium containing 2 mM glutamine, 10 mM 4-(2-hydroxyerhyl)piperazine-1-erhanesulfonic acid (HEPES), 10 mM TES, 50 ng/ml ascorbic acid, 10 μg/ml insulin, 10 μg/ml transferrin, 10 ng/ml sodium selenite and 30 μg/ml endothelial cell growth supplement, FBS 10% (Gibco BRL Life Technologies, Inc., Grand Island, NY, USA).

2.2 Gene transfer and detection (Ausubel, 1992; Nuovo, 1994; Sambrook, 1989)

2.2.1 Gene: Swine growth hormone gene with promoter was cloned in plasmid pBR322 inserted in *Escherichia coli* (*E. coli*).

2.2.2 Growth of *E. coli*: Dissolve *E. coli* in 0.3 ml Luria-Bertani (LB) plus tetracycline (2 mg/ml) medium, transfer it into a tube containing 5 ml LB plus tetracycline (2 mg/ml) medium, 37 ºC overnight, then freeze the *E. coli* (amplify in several tubes before freeze to get more samples).

2.2.3 Harvesting *E. coli*: Streak an inoculum across one side of a plate, and incubate in 2 ml of LB medium at 37 ºC overnight with vigorous shaking, then centrifuge at 12,000 g for 30 seconds at 4 ºC to get cells in the precipitate.

2.2.4 Lysis of *E. coli*: *E. coli* pellet was lyzed with alkali (0.2 N NaOH, 1% SDS). DNA was redissolved in 50 ml of TE (pH 8.0) containing DNAase-free pancreatic RNAase (20 mg/ml) and stored at -20 ºC.

2.2.5 Purification of plasmid: Plasmid DNA was purified by precipitation with polyethylene glycol.

2.2.6 Transferring swine growth hormone gene into human aorta smooth muscle cells by calcium phosphate coprecipitation: ~2×10^7 cells suspended in 0.2 ml medium were seeded into a tissue culture chamber. 48 – 72 hours later, remove medium and add 0.2 ml fresh medium, then add 0.5 mg of plasmid in 0.05 ml calcium

phosphate-HEPES-buffered saline, pH 7.0. Control the chamber temperature at 23 ºC, 37 ºC and 43 ºC.

2.2.7 Gene transfer enhanced by laser: Human aorta smooth muscle cells were cultured in F12K medium containing 2 mM glutamine, 10 mM HEPES, 10 mM TES, 50 ng/ml ascorbic acid, 10 μg/ml insulin, 10 μg/ml transferrin, 10 ng/ml sodium selenite and 30 μg/ml endothelial cell growth supplement, FBS 10% (Gibco BRL Life Technologies, Inc., Grand Island, NY). GFP gene was transferred by UV excimer laser ($XeCl_2$, 308 nm excimer laser, Spectranetics CVS-300^{TM}, Spectranetics, Colorado Springs, CO) was used with a 2.0 mm diameter optical fibers, 45 mJ/mm^2 and 25 Hz for 3 minutes at 37 ºC and 43 ºC, respect, and incubated overnight at 37 ºC for the GFP gene expression. ~2 × 10^7 rat heart muscle cells suspended in 0.2 ml medium were seeded into a tissue culture chamber. Human IL-2 gene was transfected into rat heart cells and swine growth hormone gene was transfected into human aorta smooth muscle cells by calcium phosphate coprecipitation enhanced by laser. Four hours (or other time length) later, change medium. Transfected swine growth hormone and human IL-2 gene expressions were detected using an indirect ELISA.

2.2.8 Detection of swine growth hormone: 12 – 48 hours after the addition of plasmid and incubation, the amount of swine growth hormone was measured in the medium using the indirect enzyme-linked immunosorbent assay (ELISA) (Beaugrand *et al*, 2007; López *et al*, 2007; Lee *et al*, 2008).

3 Results

With the ELISA method, transfection of the swine growth hormone gene into human smooth muscle cells resulted in swine growth hormone expression by the human smooth muscle cells. At baseline incubation, the background transfection for the swine growth hormone measurement was similar for all cell groups (p=ns). Temperature elevation from 23 ºC to 37 ºC, then to 43 ºC resulted in a significant increase of gene transfer product expression ($P < 0.01$) (Figure 1). The greatest effects occurred within 10 minutes of incubation and persisted up to 30 minutes. This was significantly greater at 37 ºC with 59% rise and then greatest at 43 ºC with 218% rise compared to 23 ºC. The similar result was gotten by Western Blotting method (data not shown). In additionally, our RT-PCR detection showed that the mRNA expression of transfected of the swine growth hormone gene was increased with the temperature increasing (data not shown).

There was a significantly higher gene transfected into cell by lasing 5 minutes (Figure 2), and significantly higher when lasing plus high temperature (Figure 3). This shows that UV excimer laser enhanced the gene transfer and the combination of the elevated temperature and lasing enhanced the gene transfer.

4 Discussions

Several techniques are currently used to transfer genes into cultured cells and into various tissues and organs (Ogura, 2003; Tomanin, 2002). These include electroporation, lipofection, calcium phosphate coprecipitation and DEAE-dextran, etc. It was successful to transfer human artificial chromosome vectors into stem cells (Oshimura *et al*, 2008). It was reported that genetic modulation of

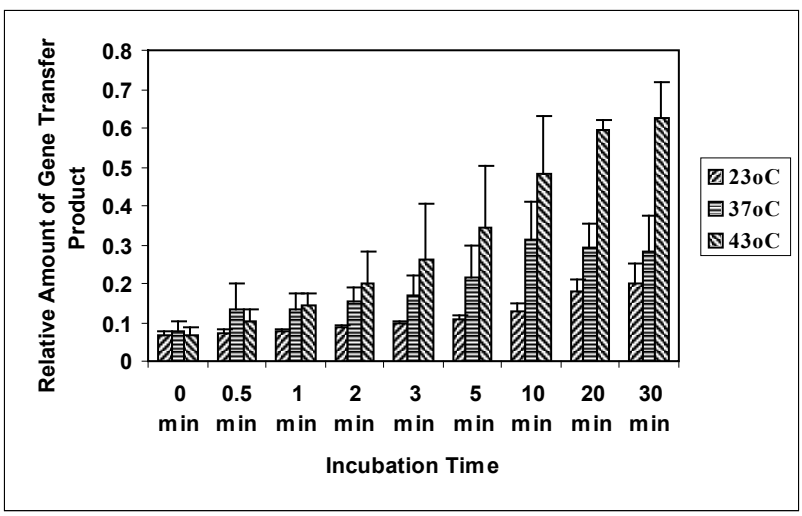

Figure 1. Increase in ambient temperature enhances swine growth hormone gene transferred into human aorta smooth muscle cells.

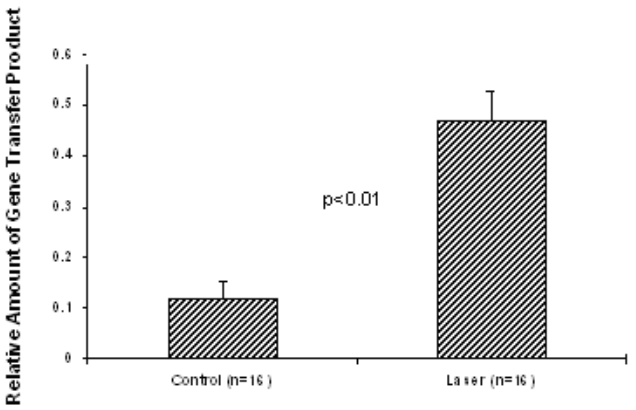

Figure 2. Laser enhanced gene transfer.

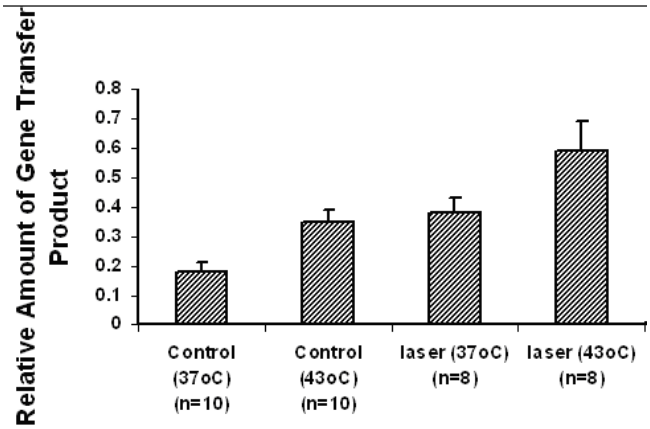

Figure 3. Combination of laser and elevated temperature on gene transfer.

heart failure using the SERCA-2a gene was associated with improvement in cardiac function and exercise capacity as well as improvements in heart-failure associated inflammatory markers (Gupta *et al*, 2008). Gene therapy has delineated putative mechanisms of disease in animal models of erectile dysfunction (Bivalacqua and Strong, 2008). Although gene therapy is a potential therapeutic approach for arterial restenosis following angioplasty, the efficiency of transfection is low regardless of the vector used. In this experiment the data demonstrate that when cultured human arterial smooth muscle cells in a chamber are heated at 43 ºC for up to 30 minutes, transient transfection by calcium phosphate coprecipitation of a plasmid expressing swine growth hormone gene was significantly enhanced. The results of our study suggest that the relatively low efficiency of gene transfer into tissues for therapy might be increased by short periods of heating during transfection. Even a few degrees of ambient temperature rise can significantly increase gene transfer into cells.

Also, in our studies, we combined the laser and elevated temperature that enhanced the gene transfer more. This is the first trial to combine the elevated temperature and laser on the gene transfection. The combination of the several gene transfer techniques could be more powerful way for the gene transfer application. This may be of value when using gene therapy with transfection procedures.

References

1. Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K. Short Protocols in Molecular Biology, Second Edition, Greene Publishing Associates, New York. 1992; Part 1: 1 – 27.
2. Bechtel J Jr, Boring JR 3rd. Antibiotic-resistance transfer in Yersinia enterocolitica. Am J Clin Pathol 1979;71:93 – 6.
3. Bivalacqua TJ, Strong TD. The use of gene transfer technology to study the pathophysiology of erectile dysfunction. J Sex Med 2008; 5(2): 268 – 75.
4. Cavazzana-Calvo M, Thrasher A, Mavilio F. The future of gene therapy. Nature 2004; 427: 779 – 81.
5. Dick JE, Sirard C, Pflumio F, Lapidot T. Murine models of normal and neoplastic human haematopoiesis. Cancer Surv 1992; 15: 161 – 81.
6. Ferreira E, Potier E, Logeart-Avramoglou D, Salomskaite-Davalgiene S, Mir LM, Petite H. Optimization of a gene electrotransfer method for mesenchymal stem cell transfection. Gene Ther 2008; 15(7): 537 – 44.
7. Goya RG, Sarkar DK, Brown OA, Herenu CB. Potential of gene therapy for the treatment of pituitary tumors. Curr Gene Ther 2004; 4(1): 79 – 87.
8. Gupta D, Palma J, Molina E, Gaughan JP, Long W, Houser S, Macha M. Improved Exercise Capacity and Reduced Systemic Inflammation after Adenoviral-Mediated SERCA-2a Gene Transfer. J Surg Res 2008; 145(2): 257 – 65.
9. Ma H, Chi C, Abela GS. Increased ambient temperature enhances human interleukin-2 gene transfer into cultured myocytes. Journal of Investigative Medicine 2004a; 52(2): S390.
10. Ma H, Chi C, Abela GS. Increase in ambient temperature enhances gene transfer into human smooth muscle cells. FASEB Journal 2004b; 18(8): C293.
11. Ma H. Technique of Animal Clone. Nat Sci 2004c; 2(1): 29 – 35.
12. Matsui T, Rosenzweig A. Targeting ischemic cardiac dysfunction through gene transfer. Curr Atheroscler Rep 2003; 5: 191 – 5.
13. Nuovo GJ. PCR in situ Hybridization: Protocol and Applications, Second Edition. Raven Press, New York. 1994; 169 – 213.
14. Ogura T. In vivo electroporation: a new frontier for gene delivery and embryology. Differentiation 2002; 70(4 – 5): 163 – 71.
15. Oshimura M, Katoh M. Transfer of human artificial chromosome vectors into stem cells. Reprod Biomed Online 2008; 16(1): 57 – 69.
16. Puchalski RB, Fahl WE. Gene transfer by electroporation, lipofection, and DEAE-dextran transfection: compatibility with cell-sorting by flow cytometry. Cytometry 1992; 13(1): 23 – 30.
17. Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning, second edition. Cold Spring Harbor Laboratory Press, New York. 1989; Part 1: 21 – 52, Part 18: 60 – 74.
18. Sapolsky RM. Gene therapy for psychiatric disorders. Am J Psychiatry 2003; 160(2): 208 – 20.
19. Temple GF, Dozy AM, Roy KL, Kan YW. Construction of a functional human suppressor tRNA gene: an approach to gene therapy for beta-thalassaemia. Nature 1982; 296: 537 – 40.
20. Terakawa M, Sato S, Ashida H, Aizawa K, Uenoyama M, Masaki

Y, Obara M. *In vitro* gene transfer to mammalian cells by the use of laser-induced stress waves: effects of stress wave parameters, ambient temperature, and cell type. J Biomed Opt 2006; 11(1): 14 – 26.
21. Tomanin R, Friso A, Alba S, Piller Puicher E, Mennuni C, La Monica N, Hortelano G, Zacchello F, Scarpa M. Non-viral transfer approaches for the gene therapy of mucopolysaccharidosis type II (Hunter syndrome). Acta Paediatr Suppl 2002; 91(439): 100 – 4.
22. Young AT, Lakey JR, Murray AG, Moore RB. Gene therapy: a lipofection approach for gene transfer into primary endothelial cells. Cell Transplant 2002; 11(6): 573 – 82.
23. Lee SH, Jaekal J, Bae CS, Chung BH, Yun SC, Gwak MJ, Noh GJ, Lee DH. Enzyme-linked immunosorbent assay, single radial immunodiffusion, and indirect methods for the detection of failure of transfer of passive immunity in dairy calves. J Vet Intern Med 2008; 22(1): 212 – 8.
24. López L, Venteo A, Aguirre E, García M, Rodríguez M, Amusátegui I, Tesouro MA, Vela C, Sainz A, Rueda P. Development of a sensitive and specific indirect enzyme-linked immunosorbent assay based on a baculovirus recombinant antigen for detection of specific antibodies against Ehrlichia canis. J Vet Diagn Invest 2007; 19(6): 635 – 42.
25. Beaugrand J, Gebruers K, Ververken C, Fierens E, Dornez E, Goddeeris BM, Delcour JA, Courtin CM. Indirect enzyme-antibody sandwich enzyme-linked immunosorbent assay for quantification of TAXI and XIP type xylanase inhibitors in wheat and other cereals. J Agric Food Chem 2007; 55(19): 7682 – 8..