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Transdermal delivery of a readthrough-inducing drug: a new approach of gentamicin administration for the treatment of nonsense mutation-mediated disorders

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To induce the readthrough of premature termination codons, aminoglycoside antibiotics such as gentamicin have attracted interest as potential therapeutic agents for diseases caused by nonsense mutations. The transdermal delivery of gentamicin is considered unfeasible because of its low permeability through the dermis. However, if the skin permeability of gentamicin could be improved, it would allow topical application without the need for systemic delivery. In this report, we demonstrated that the skin permeability of gentamicin increased with the use of a thioglycolate-based depilatory agent. After transdermal administration, the readthrough activity in skeletal muscle, as determined using a *lacZ/luc* reporter system, was found to be equivalent to systemic administration when measured in transgenic mice. Transdermally applied gentamicin was detected by liquid chromatography-tandem mass spectrometry in the muscles and sera of mice only after depilatory agent-treatment. In addition, expansion of the intercellular gaps in the basal and prickle-cell layers was observed by electron microscopy only in the depilatory agent-treated mice. Depilatory agent-treatment may be useful for the topical delivery of readthough-inducing drugs for the rescue of nonsense mutation-mediated genetic disorders. This finding may also be applicable for the transdermal delivery of other pharmacologically active molecules.

Keywords: Gentamicin/nonsense mutation/readthrough/thioglycolates/transdermal drug delivery.

Abbreviations: TEM, transmission electron microscopy; TCA, trichloroacetic acid.

More than 1800 distinctly inherited human diseases are caused by a single gene that carries nonsense mutations (*1*). In *Duchenne* muscular dystrophy (DMD), up to 20% of patients carry nonsense mutations (*2*). Similarly, up to 10% of patients (>50% in Israel) with cystic fibrosis (CF) have nonsense mutations in the CF transmembrane regulator gene (*3*). Nonsense mutations in tumour-suppressor genes are also common during the development and progression of cancer (*4*). Despite advances in gene therapy, clinical success is pending. One limitation involves the inducing genes used in the therapy, which are often targeted by the patient's autoimmune system in response to the viral proteins encoded in the vector. An alternative, pharmacologic approach to induce translational readthrough involves blocking the nonsense mutations using antibiotics. It has been reported that aminoglycoside antibiotics can interfere with the fidelity of the translation machinery. Aminoglycosides cause extensive miscoding of the mRNA *in vitro* (*5*) and allow readthrough of premature termination codons, as demonstrated in *Escherichia coli* (*6*), tetrahymena (*7*), wheat embryos (*8*), yeast (*9*), cultured mouse cells (*10*) and human cells (*11–14*).

The *mdx* mouse, which is a naturally occurring animal model for DMD, carries a point mutation (from CAA to TAA) at position 3185 in exon 23 of the dystrophin gene. Barton-Davis *et al.* (1999) reported that gentamicin restored functional dystrophin in the *mdx* mouse (*15*). Moreover, clinical trials involving patients with DMD or CF caused by nonsense mutations have shown that aminoglycosides suppress premature termination mutations in some cases (*16*, *17*).

Gentamicin is one of the most commonly used aminoglycoside antibiotics, and it is usually administered by intramuscular injection. However, it cannot be administered either orally or transdermally as it is a polarized water-soluble compound with very poor intestinal and dermal permeability. Less painful and simpler methods of gentamicin administration are needed to improve the care of patients with genetic disorders caused by nonsense mutations. To this end, transdermal drug delivery has several advantages: it: (i) bypasses gastrointestinal incompatibility and the hepatic 'first-pass' effect; (ii) reduces side-effects due to the optimization of the blood concentration-time profile; (iii) involves patient-activated/patient-modulated delivery which enhances patient compliance; and (iv) enhances target specificity (*18*). However, a major limitation of transdermal administration is the difficulty associated with delivering gentamicin through the skin barrier.

The objective of the present study was to evaluate the *in vivo*, chemically enhanced transdermal delivery of gentamicin as a readthrough-inducing drug. To this end, we established a novel transgenic mouse strain, named READ (Readthrough Evaluation and Assessment by Dual-reporter), that carries a dual-reporter gene composed of the *lacZ* and *luc* genes connected with a premature termination codon. In this system, only β-galactosidase can be translated without transcriptional readthrough, however, both enzymes would be translated when readthrough occurs. In addition, liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to validate the effect of a depilatory agent on the *in vivo* permeation of gentamicin. Finally, ultrastructural studies using electron microscopy were performed to provide insight into the potential mechanism of barrier alterations in depilatory agent-treated skin.

Materials and Methods

Chemicals

Gentamicin solution (Gentacin injection) and cream (0.1% Gentacin cream) were purchased from Schering-Plough K. K. (Osaka, Japan). Hair removal gel mousse was obtained from Reckitt Benckiser Co., Ltd. (Tokyo, Japan). All other chemicals were purchased from Sigma Chemical Co. (St Louis, MO, USA), Wako Pure Chemical Industries (Osaka, Japan) and Promega (Madison, WI, USA).

Animals

A dual-reporter transgenic mouse strain on a C57/BL6 background was generated by the Bioindustry Division of Oriental Yeast Co., Ltd (Tokyo, Japan). The dual-reporter gene consisted of the genes encoding β-galactosidase and luciferase connected with the premature termination codon 'Opal (TGA)' region (a 27-mer that contains the sequence surrounding the premature termination codon of exon 23 of the *mdx* gene for mouse dystrophin; TTGAAAGAG CAATAAAATGGCTTCAAC), and was driven by a cytomegalovirus/β-actin hybrid promoter (Fig. 1). Transgene DNA was injected into the male pronuclei of fertilized eggs which were then incubated at 37°C and transferred into the uteri of pseudopregnant ICR recipient female mice. Founder mice bearing the transgene were identified by PCR analysis of the DNA isolated from partially excised tails. Homozygous transgenic mice were obtained after crossing heterozygous littermates.

Male hairless (HR1) and normal (C57/BL6) mice (5 weeks old; ~25 g body weight) were obtained from Japan SLC, Inc. The mice were housed individually under controlled conditions of temperature and humidity and had free access to water and food. The mice were procured after approval for the present study from the University of Tokyo Animal Ethics Committee.

Skin treatment and readthrough analysis

A thioglycolate-based depilatory cream was applied for 1 min to the mouse skin, which was then rinsed with warm water to remove the cream. Gentamicin cream (1 mg gentamicin/day) was then applied and rubbed gently onto the skin daily for 7 days. At the completion of the treatment, the mice were euthanized with an overdose of ether. Tissue samples were collected from the latissimus dorsi, gluteus major, femoris lateralis, biceps femoris and quadriceps femoris. Dissected tissues were minced with scissors and homogenized in three volumes of the reporter lysis buffer (Promega) with thin glass fragments using a tissue grinder (Physcotron, Niti-on, Japan). Tissue homogenates were subjected to one round of freeze-thawing. For the readthrough assay, the lysate supernatants were collected after centrifugation at 17710*g* for 10 min, and then analyzed using the Beta-Glo and Bright-Glo luciferase assay systems (Promega).

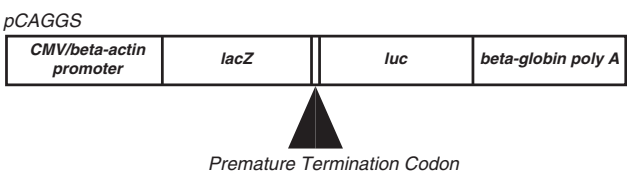

Fig. 1 Schematic structure of the dual-reporter gene construct. The dual reporter was composed of the *lacZ* and *luc* genes connected with a premature termination codon region derived from exon 23 of the *mdx* mouse dystrophin gene and driven by a cytomegalovirus/β-actin hybrid promoter. Although the premature termination codon was originally TAA, we used TGA in the present study.

The β-galactosidase and luciferase activities were measured according to the manufacturers' instructions using a luminometer (Luminescencer-JNRII, AB-2300; Atto, Japan). The readthrough efficiency was determined as the ratio of luciferase activity to β-galactosidase activity.

Liquid chromatography-tandem mass spectrometry

To trace the gentamicin introduced into the mice, LC-MS/MS analysis was performed using the 1100 Quaternary HPLC System (Agilent Technologies, USA) coupled to the API 5000 (Applied Biosystems, CA, USA). Gentamicin solution (10 ng/ml in 5% TCA), 5% TCA and blank mouse serum or muscle tissue extract in reporter lysis buffer were added in equal quantities to serve as the gentamicin standard samples. To prepare the samples for LC-MS/MS, the supernatant of the serum or muscle tissue extract was mixed with 5% TCA (twice the volume of the sample). Chromatographic separation was performed on an XTerra column (3.5 μm, 2.1 mm × 50 mm, Waters, MA, USA) maintained at 30°C in a column oven. Five millimolar hepatafluorobutyric acid (solvent A) and acetonitrile (solvent B) were used as the mobile phase under the condition of 10% B (0–2 min), 10–30% B (2–4 min, linear gradient), 30% B (4–10 min), 10–30% B (10–12 min, linear gradient), and 10% B (12–15 min) at a flow rate of 0.2 ml/min with an injection volume of 10 μl. The parent to product ion transitions for gentamicin (m/z 478.39→322.30 as gentamicin C1) was monitored on a triple quadrupe mass spectrometer, operating in the multiple reaction monitoring (MRM) with a positive ion mode of electrospray ionization (ESI) and a data collection time of 15 min. The relative quantitative value was calculated using the single-level, calibration-curve method.

Histochemical and electron microscopic analyses

Serial fresh-frozen sections (10 μm thickness) from the whole body of a mouse were prepared according to Kawamoto's film method (*19*). The whole-body sections were freeze-dried and then incubated in X-Gal solution (Nakarai, Japan) overnight at 37°C, and then placed in PBS. The adjacent sections were stained with Hematoxylin and Eosin.

For TEM analysis, small pieces (10 mm × 10 mm) of the dorsal side of the skin were excised from the hairless mice with a single-edge razor blade. To prepare ultra-thin sections for electron microscopy, tissues were doubly fixed with 2% glutaraldehyde in 0.1 M phosphate buffer and 1% osmium tetroxide (in 0.1 M phosphate buffer), and then dehydrated with a graded series of ethanol. The tissues were then embedded in epoxy resin. Ultra-thin sections were stained with uranyl acetate and lead citrate and observed under an electron microscope (H-7500; Hitachi, Tokyo, Japan) at an acceleration voltage of 100 kV. The images were optimized for contrast and brightness using Photoshop CS3 software (Adobe Inc., San Jose, USA).

Results

Establishment of a transgenic mouse for transcriptional readthrough evaluation

In order to measure transcriptional readthrough activity, we established a novel transgenic mouse strain, named READ, which expressed a dual-reporter gene. The dual reporter construct was composed of the *lacZ* and *luc* genes connected with a premature termination codon region (Fig. 1). Although the premature termination codon of the *mdx* mouse was originally TAA, we adopted TGA-centered sequences because

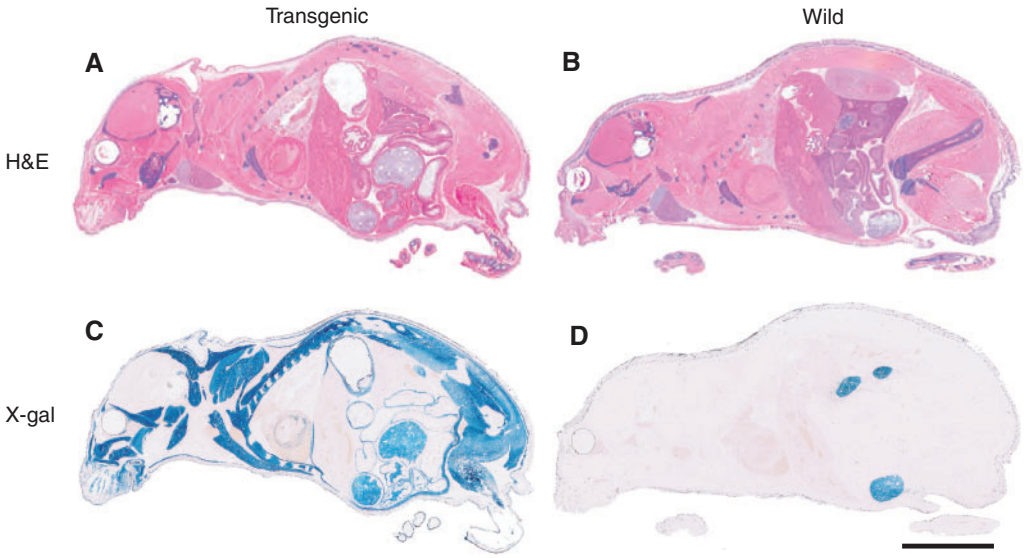

Fig. 2 Whole-body X-gal staining of a transgenic mouse. Whole-body fresh sections of 5-week-old transgenic (A and C) and control (B and D) mice were stained with Hematoxylin-Eosin (A and B) and X-gal (C and D). The transgenic mouse expressed β-galactosidase in the skeletal and cardiac muscles. The contents of the digestive tracts stained positive with X-gal due to the native bacterial β-galactosidase of flora (D). Bar = 1 cm.

aminoglycoside antibiotics tend to exhibit the highest readthrough activity for TGA (*20*). When a test substance without readthrough activity was administered to READ mice, only β-galactosidase was translated. We confirmed β-galactosidase expression in striated muscles, including the diaphragm and heart, by X-gal staining of whole-body sections (Fig. 2C). The readthrough efficiency was determined as the ratio of luciferase activity to β-galactosidase activity.

Readthrough activity of gentamicin in READ mice

The readthrough activities of gentamicin following single, daily subcutaneous injections (1 mg in 0.1 ml, $n = 6$) and transdermal administration (1 mg in 1 g, $n = 5$) after depilatory treatment were compared after 7 days. The mice used for transdermal administration were treated with depilatory cream on day zero. A commercially available topical cream containing 0.1% gentamicin was then applied daily to the skin of the back, hip and thigh of each transgenic mouse. Gentamicin applied to the depilatory agent-treated skin induced readthrough in the muscle tissues at the same level as that observed after subcutaneous injection (Fig. 3). Transdermally administered gentamicin resulted in readthrough activity only in depilatory agent-treated READ mice, but not in untreated ones.

Detection of gentamicin in muscle and serum of subcutaneously and transdermally treated mice

To study the permeability of gentamicin into the muscle tissues, LC-MS/MS analysis was performed. The presence of gentamicin C1 was confirmed in both the subcutaneously and transdermally treated groups (Fig. 4). The gentamicin components C1a and C2 were also detected in the same manner (data not shown). To evaluate whether pretreatment with the thioglycolate-based depilatory agent enhances gentamicin permeation through the skin, the detection of

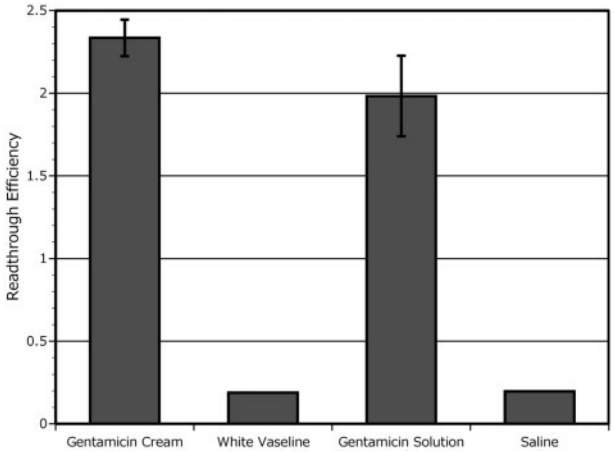

Fig. 3 Effects of topical and subcutaneous administration of gentamicin on readthrough activity in READ mice. The readthrough activity of gentamicin was compared between mice that were treated by daily topical administration (1 mg in 1 g, $n = 5$) onto depilatory-treated skin and those treated by subcutaneous injection (1 mg in 100 µl, $n = 6$) for 7 days. The data for gentamicin cream administration showed the same increase in readthrough efficiency as that observed for subcutaneous injection (gentamicin solution). Error bars indicate SDs.

gentamicin in the sera and muscle tissue extracts of hairless mice was carried out using LC-MS/MS. We confirmed that the LC-MS/MS peak observed for the depilatory-treated group was in the identical position as that of the reference material, whereas the peak associated with gentamicin C1 was undetectable in the untreated group (Fig. 5). The results indicate that the depilatory agent significantly increased the absorption of gentamicin by the skin.

Ultrastructure of depilatory agent-treated skin

To examine whether ultrastructural changes in the skin were caused by treatment with the depilatory agent,

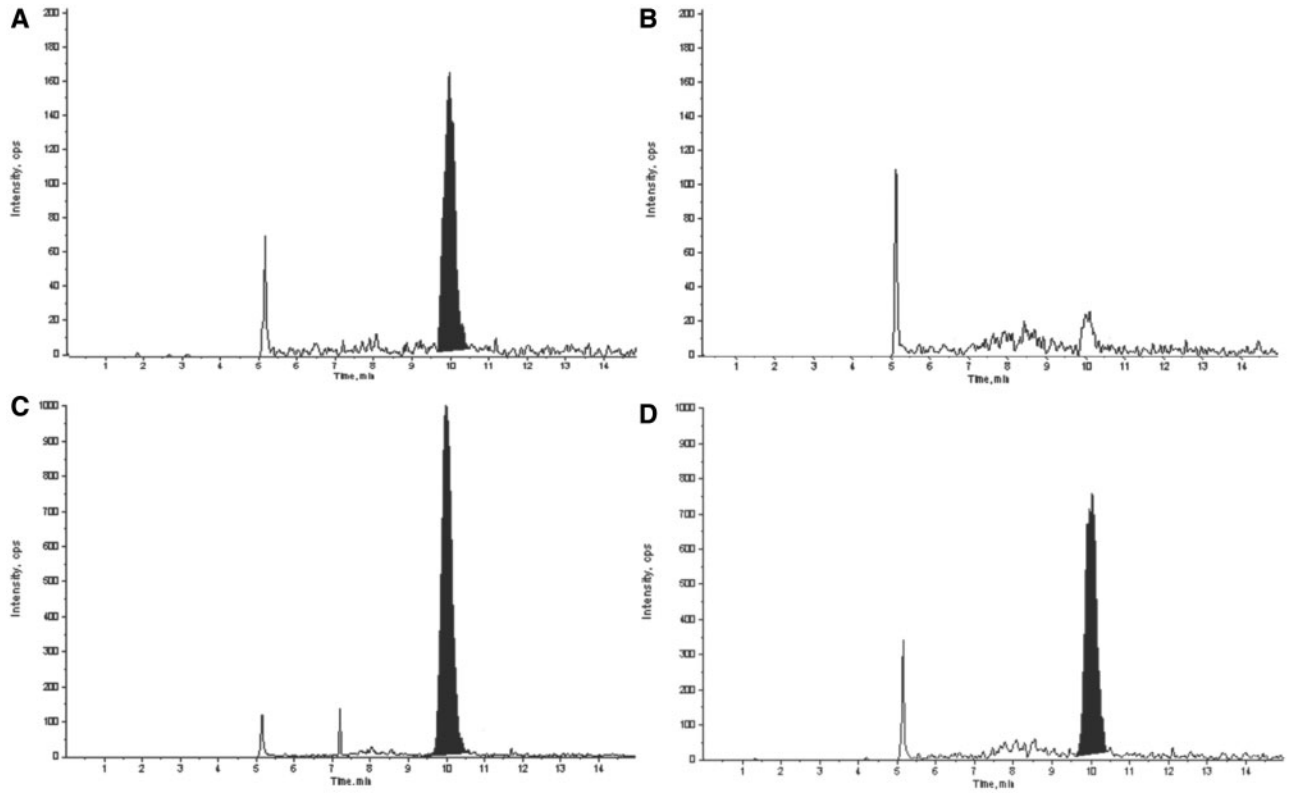

Fig. 4 LC-MS/MS detection of gentamicin in the muscle tissue extracts from gentamicin-treated mice. The mass chromatograms of gentamicin C1 in muscle tissue extracts from C57/BL6 mice: gentamicin standard sample (A); no administration (B); subcutaneous injection of gentamicin daily for 7 days (C); transdermal administration of gentamicin cream daily for 7 days after one-time depilatory treatment (D). The major (black-painted) peak obtained for both administration groups was identical to the gentamicin standard sample.

TEM analysis was performed with control, 1 min- and 24 h-treated samples. In the non-treated control sample, the cells were closely packed together and intercellular spaces were not observed (Fig. 6A). However, even after just 1 min of treatment with the depilatory agent, the formation of gaps could be observed when the control (Fig. 6A) and experimental sample (Fig. 6B) were compared. In the sample treated 24 h with the depilatory agent, a large expansion of the intercellular gaps and extraordinary spaces in the basal and prickle-cell layers were clearly evident (Fig. 6C).

Discussion

Establishment of a transgenic mouse for the in vivo assay of readthrough activity

We established and demonstrated the efficacy of a dual-reporter transgenic mouse strain, named READ, which can be used for the detection of readthrough activity *in vivo* for the first time. It is difficult to measure the amount of dystrophin quantitatively because it is relatively large (molecular weight of 427 kDa) and is only present in small amounts in striated muscles. Therefore, the reporter assay with READ mouse is quantitative and efficient in comparison to the detection of dystrophin using a *mdx* mouse. In rare cases, some luciferase inhibitors, a compound such as PTC124, acting through post-translational *luc* reporter stabilization, appear to activate gene expression (*21*). We consider the importance of implementing the appropriate control assays. The READ mouse assay system used in this study makes it possible not only to screen new molecules which induce readthrough, but also to examine the pharmacokinetics and side effects associated with such molecules. This system can also be used for the optimization of various routes of drug administration. Our READ mouse provides a powerful and valuable tool for the development of novel readthrough therapeutics.

Depilatory agent-treatment enhances dermal permeability and is useful for transdermal drug delivery

Transdermal drug delivery systems offer many advantages over conventional dosage forms, such as improved patient compliance, reduced side effects, no hepatic first pass effects, and the possibility to easily interrupt or terminate treatment (*22–24*). Moreover, compared with oral administration, such a non-invasive drug delivery route significantly reduces drug degradation due to the lower metabolic activity at the site of administration. It also bypasses hepatic circulation which is a major site of potential drug metabolism (*25*). However, given the low permeability of external molecules, such as gentamicin, the skin remains a minor portal of entry for drugs (*26*). Therefore, various approaches aimed at decreasing the resistance of skin to drug penetration have been investigated (*27*). The effects of a depilatory agent on the percutaneous absorption of testosterone and theophylline in Guinea pigs (*28*) and rats (*29*), respectively, and on the

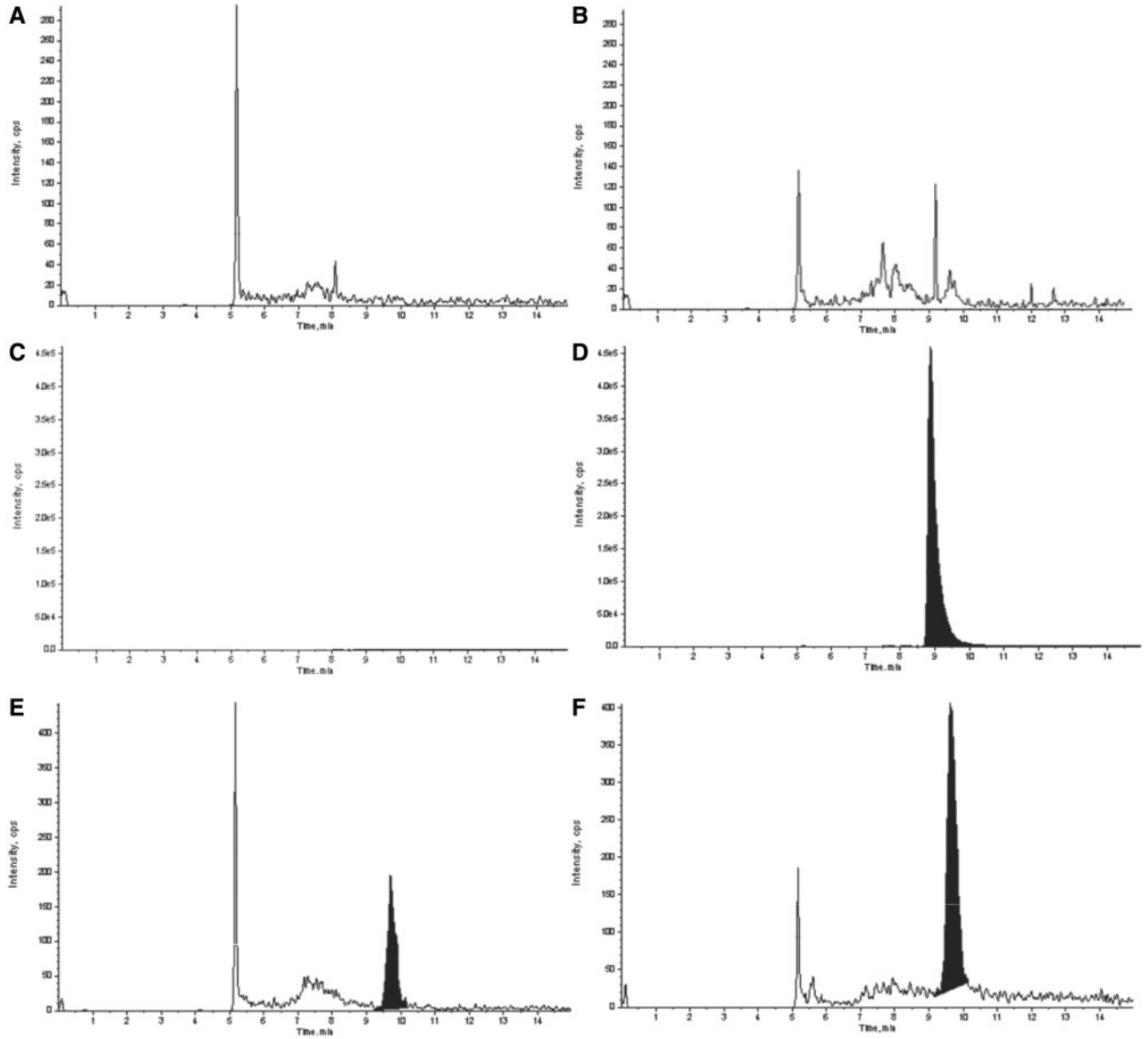

Fig. 5 LC-MS/MS detection of gentamicin in the sera and muscle tissue extracts of hairless mice with or without depilatory treatment. Mass chromatograms of gentamicin C1 in the sera (A, C and E) and muscle tissue extracts (B, D and F) from hairless mice: non-depilatory-treated and gentamicin-administrated daily for 7 days (A and B); one-time depilatory-treated 24 h prior to administration and gentamicin-administrated daily for 7 days (C and D); part of (C) at a higher magnification (E); gentamicin standard sample (F). The black-painted peak showed the existence of gentamicin. Gentamicin was not detected in the non-depilatory-treated group, whereas gentamicin was present in the depilatory-treated-group, as assessed by comparison with the reference material in LC-MS/MS.

ionotophoretic delivery of insulin in diabetic rats (*30*, *31*), porcine epidermis (*32*, *33*), and on the human stratum corneum (*34*) have been reported. As shown in Fig. 3, topically applied gentamicin cream induced the readthrough of a premature termination codon and was as effective as subcutaneous injection.

Presence of transdermally delivered gentamicin in muscle and serum

To determine whether a depilatory agent could influence the permeability of skin, we next investigated the permeation of gentamicin following transdermal administration using a validated LC-MS/MS system (Fig. 4). The quantity of gentamicin was calculated from the peak area and when appropriate, we normalized the measured levels to a peak area of known concentration in a gentamicin standard sample. In the muscle tissue extracts, the concentration of gentamicin was ~2-fold higher on average ($n = 3$) for transdermal administration when compared to subcutaneous injections. It is likely that transdermal administration can extend the duration of treatment with gentamicin, which has a short half-life.

The administered gentamicin was present in both the sera and muscle tissue extracts of depilatory agent-treated mice (Fig. 5). Furthermore, LC-MS/MS results indicated that the amount of gentamicin was significantly higher (~7000-fold) in the muscle tissue than in the serum. *In vivo* transdermal absorption experiments demonstrated that the depilatory agent drastically reduced the barrier function of the skin

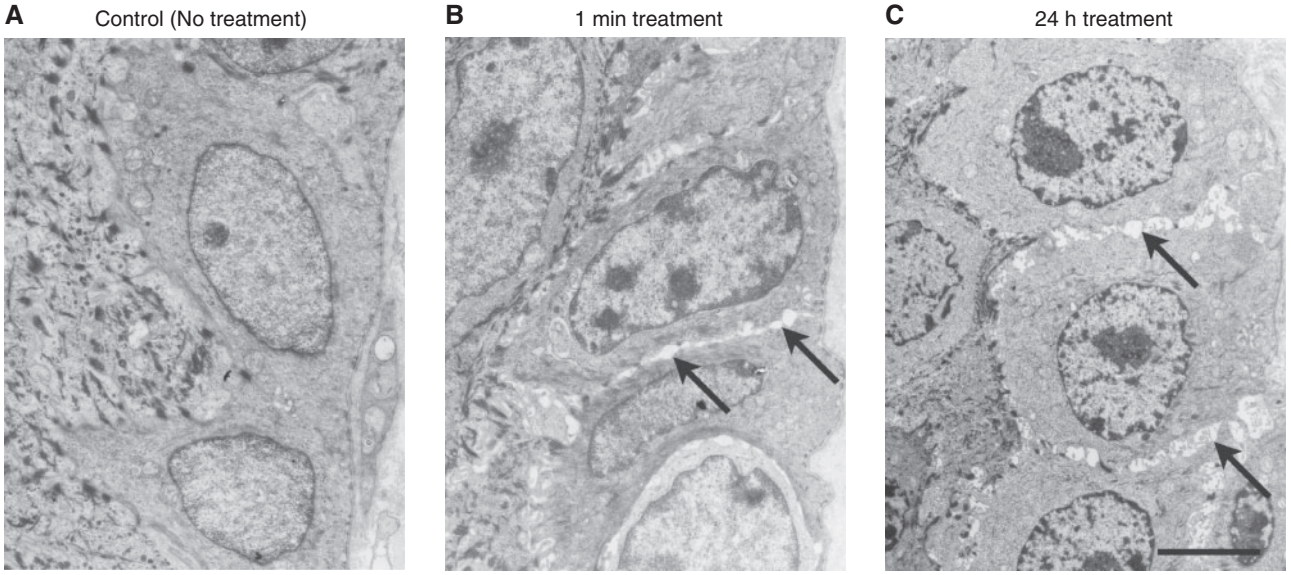

Fig. 6 Electron micrographs of skin samples from hairless mice treated with a depilatory cream. Dorsal skin samples from 5-week-old hairless mice; control (non-depilatory-treated) (A); treated with the depilatory cream for 1min (B); and treated with depilatory cream for 24 h during the study (C). Expansion of the intercellular gaps in the basal and prickle-cell layers caused by depilatory processing was evident (B and C, arrows). Bar = 5 μm.

and exerted a direct penetration-enhancing effect on the transdermal route. However, these may be based on the difference in hairless mouse and normal hairy mouse. The hairless mice have been widely and frequently used to predict the effects of penetration enhancers in human skin. Nevertheless, the information on the suitability of hairless mice in percutaneous penetration is not uniform and sometimes contradictory (*35*). The skin of hairless mice is obviously thin in comparison with normal mice and has many folds. Moreover, because the skin penetration is a complex process, a judgement about this penetration-enhancing effect on hairless mouse skin must be cautious. In addition, we can conclude that differences in the delivery vehicles affect the penetration and bioavailability of the drug. Specifically, the effectiveness of gentamicin in an emulsion base (i.e. a cream) was higher than in oils and fats as determined by the readthrough activity and the LC-MS/MS analysis (data not shown).

Depilatory agent-treatment causes ultrastructural changes in the skin

To gain further insight into the mechanisms by which depilatory agents affect skin permeability, we evaluated ultrastructural changes in the skin caused by a thioglycolate-based depilatory agent (Fig. 6). As compared to the non-treated control, both the 1 min- and 24 h-treatments disrupted the structural integrity of the basal and prickle-cell layers. Jin-Ning Lee and colleagues (*34*) have shown that depilatory agents enhance transepidermal drug delivery by reducing the resistance of both the transcellular and intercellular routes of the stratum corneum. Our present results indicate that the alteration and expansion of the intracellular spaces in the basal and prickle-cell layers could be due to the shrinkage of cells in those layers, which in turn leads to reduced resistance.

Readthrough for rescuing muscular dystrophy

In this study, we selected gentamicin to evaluate the efficacy of topical drug delivery. Aminoglycosides have emerged as vanguard pharmacogenetic agents for the treatment of human genetic disorders due to their ability to suppress translation termination caused by nonsense mutations. Gentamicin injections into *mdx* mice, a model for DMD, restored dystrophin in up to 20% of the muscle fibres and ameliorated the clinical symptoms of diseases (*15*). As gentamicin can cause serious side effects, such as inner ear and kidney toxicities, and may generate resistant bacteria, non-aminoglycoside, readthrough-inducing molecules are being actively sought. Previously, we reported that the dipeptide antibiotic negamycin has the ability to suppress nonsense mutations with lower toxicity than gentamicin (*36–38*). Thus, negamycin and its derivatives represent therapeutic candidates for genetic disorders, and are a topic for future studies. The small-molecular agent PTC124 (also known as Ataluren) has also been reported as a promising readthrough drug for DMD patients with nonsense mutations (*39*). Our transgenic mouse would be applicable to investigate the potential *in vivo* readthrough activity of these and other new molecules.

In the present study, we demonstrated that a transdermally delivered readthrough drug promotes the bypassing of a premature termination codon, which represents a novel approach for the treatment of genetic diseases caused by nonsense mutations. The transdermal delivery of drugs involves the continuous administration of therapeutic molecules through the skin, and has many advantages including the maintenance of low plasma drug levels and improving patient

compliance. We also showed the effectiveness of a thioglycolate-based depilatory agent to enhance the topical delivery of gentamicin. This finding may also be applicable for the transdermal delivery of other pharmacologically active molecules.

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Conflict of interest

None declared.

References

1. Kellermayer, R. (2006) Translational readthrough induction of pathogenic nonsense mutations. *Eur. J. Med. Genet.* **49**, 445–450
2. Diop, D., Chauvin, C., and Jean-Jean, O. (2007) Aminoglycosides and other factors promoting stop codon readthrough in human cells. *C. R. Biol.* **330**, 71–79
3. Kerem, E., Hirawat, S., Armoni, S., Yaakov, Y., Shoseyov, D., Cohen, M., Nissim-Rafinia, M., Blau, H., Rivlin, J., Aviram, M., Elfring, G., Northcutt, V., Miller, L., Kerem, B., and Wilschanski, M. (2008) Effectiveness of PTC124 treatment of cystic fibrosis caused by nonsense mutations: a prospect phase II trial. *Lancet* **372**, 719–727
4. Kaufman, R. (1999) Correction of genetic disease by making sense from nonsense. *J. Clin. Invest.* **104**, 367–368
5. Davies, J., Gorini, L., and Davis, B.D. (1965) Misreading of RNA codewords induced by aminoglycoside antibiotics. *Mol. Pharmacol.* **1**, 93–106
6. Gorini, L. (1974) Ribosomes (Nomura, M., Tissieres, T., and Lengyel, P., eds), pp. 791–803, Cold Spring Harbor Laboratory Press, NY
7. Palmer, E. and Wilhelm, J.M. (1978) Mistranslation in a eukaryotic organism. *Cell* **13**, 329–334
8. Wilhelm, J.M., Jessop, J.J., and Pettitt, S.E. (1978) Aminoglycoside antibiotics and eukaryotic protein synthesis: stimulation of errors in the translation of natural messengers in extracts of cultured human cells. *Biochemistry* **17**, 1149–1153
9. Palmer, E., Wilhelm, J.M., and Sherman, F. (1979) Phenotypic suppression of nonsense mutants in yeast by aminoglycoside antibiotics. *Nature* **277**, 148–150
10. Sangkuhl, K., Schulz, A., Rompler, H., Yun, J., Wess, J., and Schoneberg, T. (2004) Aminoglycoside-mediated rescue of a disease-causing nonsense mutation in the V2 vasopressin receptor gene *in vitro* and *in vivo*. *Hum. Mol. Genet.* **13**, 893–903
11. Wilhelm, J.M., Jessop, J.J., and Pettitt, S.E. (1978) Aminoglycoside antibiotics and eukaryotic protein synthesis: stimulation of errors in the translation of natural messengers in extracts of cultured human cells. *Biochemistry* **17**, 1149–1153
12. Lai, C.H., Chun, H.H., Nahas, S.A., Mitui, M., Gamo, K.M, Du, L., and Gatti, R.A. (2004) Correction of ATM gene function by aminoglycoside-induced read-through of premature termination codons. *Proc. Natl Acad. Sci. USA* **101**, 15676–15681
13. Schulz, A., Sangkuhl, K., Lennert, T., Wigger, M., Price, D.A., Nuuja, A., Gruters, A., Schultz, G., and Schoneberg, T. (2002) Aminoglycoside pretreatment partially restores the function of truncated V(2) vasopressin receptors found in patients with nephrogenic diabetes insipidus. *J. Clin. Endocrinol. Metab.* **87**, 5247–5257
14. Xi, B., Guan, F., and Lawrence, D.S. (2004) Enhanced production of functional proteins from defective genes. *J. Am. Chem. Soc.* **126**, 5660–5661
15. Barton-Davis, E.R., Cordier, L., Shoutourma, D.I., Leland, S.E., and Sweeney, H.L. (1999) Aminoglycoside antibiotics restore dystrophin function to skeletal muscles of *mdx* mice. *J. Clin. Invest.* **104**, 375–381
16. Politano, L., Nigro, G., Nigro, V., Piluso, G., Papparella, S., Paciello, O., and Comi, L.I. (2003) Gentamicin administration in Duchenne patients with premature stop codon. Preliminary results. *Acta Myol.* **22**, 15–21
17. Wilschanski, M., Yahav, Y., Yaacov, Y., Blau, H., Bentur, L., Rivlin, J., Avram, M., Bdolah-Abram, T., Bebok, Z., Shushi, L., Kerem, B., and Kerem, E. (2003) Gentamicin-induced correction of CFTR function in patients with Cystic Fibrosis and CTFR stop mutations. *N. Engl. J. Med.* **349**, 1433–1441
18. Thong, H.Y., Zhai, H., and Maibach, H.I. (2007) Percutaneous penetration enhancers: an overview. *Skin Pharmacol. Physiol.* **20**, 272–282
19. Kawamoto, T. (2003) Use of a new adhesive film for the preparation of multi-purpose fresh-frozen sections from hard tissues, whole-animals, insects and plants. *Arch. Histol. Cytol.* **66**, 123–143
20. Howard, M.T., Shirts, B.H., Petros, L.M., Flamigan, K.M., Gesteland, R.F., and Atkins, J.F. (2000) Sequence specificity of aminoglycoside-induced stop codon readthrough: potential implications for treatment of Duchenne muscular dystrophy. *Ann. Neurol.* **48**, 164–169
21. Auld, D.S., Thorne, N., Maguire, W.F., and Inglese, J. (2009) Mechanism of PTC124 activity in cell-based luciferase assays of nonsense codon suppression. *Proc. Natl Acad. Sci. USA* **106**, 3585–3590
22. Balfour, J.A. and Heel, R.C. (1990) Transdermal estradiol. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in the treatment of menopausal complaints. *Drugs* **40**, 561–582
23. Marjukka Suhonen, T., Bouwstra, J.A., and Urtti, A. (1999) Chemical enhancement of percutaneous absorption in relation to stratum corneum structural alterations. *J. Control Release* **59**, 149–161
24. Cevc, G. (2003) Transdermal drug delivery of insulin with ultradeformable carriers. *Clin. Pharmacokinet.* **42**, 461–474
25. Roy, S. (1999) Preformulation aspects of transdermal drug delivery systems in *Transdermal and Topical Drug Delivery Systems* (Ghosh, T., Pfister, W., and Yum, S., eds), pp. 139–166, Interpharm, Buffalo Drove, IL
26. Wester, R. and Maibach, H. (1992) Percutaneous absorption of drugs. *Clin. Pharmacokinet.* **23**, 235–266
27. Williams, A.C. and Barry, B.W. (2004) Penetration enhancers. *Adv. Drug Deliv. Rev.* **56**, 603–618
28. Andersen, K.E., Maibach, H.I., and Anjo, M.D. (1980) The guinea-pig: an animal model for human skin absorption of hydrocortisone, testosterone and benzoic acid. *Br. J. Dermatol.* **102**, 447–453
29. Kushida, K., Masaki, K., Matsumura, M., Ohshima, T., Yoshikawa, H., Takada, K., and Muranishi, S. (1984)

Application of calcium thioglycolate to improve transdermal delivery of theophylline in rats. *Chem. Pharm. Bull.* **32**, 268–274
30. Zakzewski, C., Wasilewski, J., Cawley, P., and Ford, W. (1998) Transdermal delivery of regular insulin to chronic diabetic rats: effect of skin preparation and electrical enhancement. *J. Control Release* **50**, 267–272
31. Kanikkannan, N., Singh, J., and Ramarao, P. (1999) Transdermal iontophoretic delivery of bovine insulin and monomeric human insulin analogue. *J. Control Release* **59**, 99–105
32. Rastogi, S. and Singh, J. (2003) Passive and iontophoretic transport enhancement of insulin through porcine epidermis by depilatories: permeability and fourier transform infrared spectroscopy studies. *AAPS PharmSciTech* **4**, E29
33. Rastogi, S. and Singh, J. (2004) Iontophoretic enhancement of leuprolide acetate by fatty acids, limonene, and depilatory lotions through porcine epidermis. *Pharm. Dev. Tech.* **9**, 341–348
34. Lee, J.-N., Jee, S.-H., Chan, C.-C., Lo, W., Dong, C.-Y., and Lin, S.-J. (2008) The effects of depilatory agents as penetration enhancers on human stratum corneum structures. *J. Invest. Dermatol.* **128**, 2240–2247
35. Simon, G.A. and Maibach, H.I. (1998) Relevance of hairless mouse as an experimental model of percutaneous penetration in man. *Skin Pharmacol. Appl. Skin Physiol.* **11**, 80–86
36. Arakawa, M., Shiozuka, M., Nakayama, Y., Hara, T., Hamada, M., Kondo, S., Ikeda, D., Takahashi, Y., Sawa, R., Nonomura, Y., Sheykholeslami, K., Kondo, K., Kaga, K., Kitamura, T., Suzuki-Miyagoe, Y., Takeda, S., and Matsuda, R. (2003) Negamycin restores dystrophin expression in skeletal and cardiac muscles of *mdx* mice. *J. Biochem.* **134**, 751–758
37. Allamand, V., Bidou, L., Arakawa, M., Floquet, C., Shiozuka, M., Paturneau-Jouas, M., Gartioux, C., Butler-Browne, G., Mouly, V., Rousset, J.P., Matsuda, R., Ikeda, D., and Guicheney, P. (2008) Drug induced readthrough of premature stop codons leads to the stabilization of laminin alpha2 chain mRNA in CMD myotubes. *J. Gene Med.* **10**, 217–224
38. Hayashi, Y., Regnier, T., Nishiguchi, S., Sydnes, M.O., Hashimoto, D., Hasegawa, J., Kato, T., Kajimoto, T., Shiozuka, M., Matsuda, R., Noda, M., and Kiso, Y. (2008) Efficient total synthesis of (+) – Negamycin, a potential chemotherapeutic agent for genetic diseases. *Chem. Comm.* **20**, 2379–2381
39. Welch, E.M., Barton, E.R., Zhuo, J., Tomizawa, Y., Friesen, W.J., Trifillis, P., Paushkin, S., Patel, M., Trotta, C.R., Hwang, S., Wilde, R.G., Karp, G., Takasugi, J., Chen, G., Jones, S., Ren, H., Moon, Y.C., Corson, D., Turpoff, A.A., Campbell, J.A., Conn, M.M., Khan, A., Almstead, N.G., Hedrick, J., Mollin, A., Risher, N., Weetall, M., Yeh, S., Branstrom, A.A., Colacino, J.M., Babiak, J., Ju, W.D., Hirawat, S., Northcutt, V.J., Miller, L.L., Spatrick, P., He, F., Kawana, M., Feng, H., Jacobson, A., Peltz, S.W., and Sweeney, H.L. (2007) PTC124 targets genetic disorders caused by nonsense mutations. *Nature* **447**, 87–91